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NUTRITION REVIEWS

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THE ORGANIZATION OF NUTRITION ACTIVITIES IN AUSTRALIA

For a full appreciation of the present organization of nutrition activities in Australia it is desirable to review the food consumption pattern and the degree and extent of malnutrition in Australia. The principal source of this information is the six reports of the Advisory Council on Nutrition which was appointed by the Australian Government in 1936 to report upon "the state of nutrition of the Australian people" and "the nature of any evidence that the Australian people were undernourished."

The food consumption pattern of the Australian people is remarkably uniform, being based upon the traditional fare of England. Because of the extent of the wool industry, mutton has always been comparatively cheap and this has led to a universally high meat consumption, particularly in inland areas.

In contrast to this, fresh fruit and green vegetables are generally scarce in inland areas, owing principally to the great distances and the comparatively small populations there. Because of climatic factors and often a limited water supply, it is not possible to produce fruit and vegetables locally in many inland towns during the summer months. While canned vegetables and fruit juices would be a satisfactory substitute, the limited production of these in Australia before the present war did not ensure regular and cheap supplies of these articles to the inland towns. The tremendous development of this industry during the present war to supply the needs of the forces of the United Nations in the Southwest Pacific area will have far reaching effects upon the pattern of food consumption in Australia after the war.

It has been estimated that, based on citrus fruit production alone, the average intake of ascorbic acid in Australia would be below that necessary to supply the whole population with the Recommended Daily Allowances set by the Food and Nutrition Board of the National Research Council of America. However, when the supply of ascorbic acid from other sources, particularly from potatoes and tomatoes, is added to that from citrus fruit, the quantity is sufficient to meet the Recommended Daily Allowances. Inequalities of distribution, however, mean that the large cities and towns generally have a surplus of foods rich in ascorbic acid, while in inland areas the supply is inadequate.

The average per capita consumption of milk by the Australian population is considerably below that in the United States of America. Again because of the factors outlined above, it is frequently impossible to produce milk locally in many parts of the inland. Processed milk particularly—in recent years dried whole milk—supplements the limited supplies of fresh milk. The over-all milk production in Australia is greatly in excess of the domestic requirements. In peacetime about 50 per cent of the total milk production is consumed in Australia as milk, butter, or cheese, and the remainder is exported as processed articles.

Gross malnutrition resulting in the development of frank deficiency diseases does not, except under extremely rare circumstances, occur in Australia, although cases of minor departures from normal health due to inadequate diets do occur. It was the opinion of the Advisory Council on Nutrition that the main cause of malnutrition in Australia was faulty selection of diets, due in some instances to poverty but more frequently to ignorance.

From this brief review it is evident that, with the possible exception of rich and stable sources of ascorbic acid, the over-all production of food in Australia is sufficient to supply the population with adequate quantities of the right kind of food. The problem is mainly that of better distribution of the available supplies and a fuller appreciation by the general public of the need for a more balanced diet and a generally higher consumption of the protective foods.

The first organized study of nutritional problems in Australia was undertaken by the Advisory Council on Nutrition from 1936 to 1938. It was recommended that the work should be continued, and the majority of its members subsequently became foundation members of the Nutrition Committee of the National Health and Medical Research Council, which commenced its activities in January, 1939. The activities of the committee during the last six years can be grouped under three main headings: (a) directing research; (b) advising upon nutrition education; (c) acting as an advisory body to the Australian Government concerning matters relating to food and nutrition.

In addition to the Nutrition Committee of the National Health and Medical Research Council, there is in each State a State Nutrition Committee. The main functions of these committees are: (a) to advise the State Governments on matters relating to food and nutrition; (b) to prepare detailed information on matters relating to food and nutrition; (c) to collect data relative to food distribution; (d) to establish centers through which information can readily be disseminated.

All of these committees are advisory and with the exception of the research staff which from time to time works under the direction of the Commonwealth Committee they have not any staff directly under their control. All committees are, however, closely affiliated with the various Health Departments and it is in these departments that the work associated with the committees is carried on.

One of the most serious handicaps to the development of plans for the extension of nutrition education has been the shortage of persons who have had the necessary training. This has been due almost entirely to the fact that no Department of Home Economics exists at an Australian university. The establishment of a department in at least one Australian university is a most pressing need.

In the field of nutrition research it is evident that: (a) more information is required on the chemical composition of Australian foods; and (b) more information is required about the nutritional status of the Australian population. The policy of those responsible for the organization of nutrition activities in Australia is to collect this information as rapidly as possible.

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STARVATION AND WATER DEPRIVATION

The present world conflict has once again given great impetus to the study of some of the less spectacular, but not less dangerous hazards, which may bring disaster to the victim by altering his internal environment through changes in the external environment less precipitous than the high explosive. The perils of life and warfare in desert and jungle, at high altitudes, and below the surface of the sea have presented new problems and revived interest in many old ones. Investigations directed to their solution have been carried on in special laboratories created for such a purpose by the

armed forces and in established civilian institutions.

The problem of survival of castaways and those shipwrecked at sea is coeval with man's use of boats. In recent times it has been magnified because of enemy activity against the Navy, Merchant Marine, and Air Forces. The effects of partial or complete deprivation of food and water are therefore of great practical importance as well as matters of considerable academic interest. Some work done under the combined auspices of the Yale University School of Medicine and the Aero-Medical Laboratory, Wright Field, Dayton, Ohio, has been published. Winkler, Danowski, Elkinton, and Peters (*J. Clin. Invest.* **23,** *807 (1944)*) have reported their studies on the electrolyte, nitrogen, and water balances during water deprivation and starvation in 8 healthy young soldiers, and the effect of ingestion of fish, carbohydrate, and salt solutions. It was found that fasting caused an increase in the negative water balance during water deprivation. The loss of fluids during the early stages occurred primarily from the intracellular phase but later from the extracellular. There was no advantage in drinking a hypotonic saline mixture of fresh water and sea water, and it might have the distinct psychologic disadvantage of leading to the use of more concentrated solutions, or pure sea water when the supply of fresh water dwindled. It was found that carbohydrate reduced the negative nitrogen balance, the formation of ketone bodies, the volume of urine, and the degree of dehydration in subjects completely deprived of water. These effects derive from its protein sparing capacity and the water of oxidation produced by its combustion. It would appear that less urine is formed because less fluid is required to carry off the decreased amounts of nitrogenous end products of protein metabolism. Conservation of body water occurs with ingestion of carbohydrate even before proteinemia becomes pronounced. The ancient question of whether to eat fish when drinking water is scant or lacking has been answered in the negative, at least for haddock, since all the water content of the fish is required by the subject to excrete the nitrogenous products derived from the metabolism of fish protein. All evidence points to the contraindication of protein food when the supply of potable water is limited. It must be remembered that these studies were carried out on a few subjects only, and though the results are reasonably consistent it would be well to have more data. There is still a lively interest in the possibility that a certain amount of protein food may exert a beneficial sparing action on body protein without causing a significant increase in the water requirement.

In an extension of the study a series of experiments were made by Danowski, Elkinton, and Winkler (*J. Clin. Invest.* **23,** *816 (1944)*) on 14 fully grown dogs to ascertain the effects of a dry protein ration. It was found that dry protein fed at a level of 1.5 g. per kilogram without water would maintain dogs in nitrogen equilibrium without causing any change in the nitrogen excretion. When the protein ingested was fed at a level of 2.0 to 7.2 g. per kilogram it caused an increase in protein metabolism and an elevation of the nitrogenous end products excreted in the urine. When the excretion of nitrogen increased it required an increase in the volume of urine. This is true even in the presence of dehydration and thus any addition of dietary protein over that required to support nitrogen equilibrium increases dehydration and reduces survival time unless there is an adequate supply of fresh water. If the water supply is adequate similar quantities of protein will maintain dogs in good condition for at least four weeks. Just as in man, the dog is enabled to conserve water when carbohydrate is given, and for the same reasons. Kartin, Man, Winkler, and Peters (*Ibid.* **23,** *824 (1944)*) have made a study of the serum lipids and blood ketones of adult human

males, dogs, and monkeys during fasting, in some instances with concurrent water deprivation. In contradistinction to dogs, which developed no appreciable ketosis and no hyperlipemia, the blood ketones in the human subjects usually increased progressively during periods of starvation lasting from two to six days. As fasting was prolonged, serum lipid phosphorus rose perceptibly and there was a slight but unequivocal rise in serum cholesterol. In monkeys the behavior was more like that in man, though both blood ketones and serum lipids rose more rapidly and further. Cholesterol was most affected. There was no appreciable change in the neutral fat component. Hyperlipemia could be abolished in both man and monkeys by the administration of carbohydrate in large enough quantities to reduce or abolish the ketosis. Comparisons with the ketosis of diabetic coma show that it reaches much greater extremes than that induced by starvation of a few days. The Yale workers suggested that "the hyperlipemia of carbohydrate starvation arises not merely because a larger quantity of fat is being utilized, but because an unusually large amount of fat is being converted to ketone bodies." The authors mention, but do not stress, the fact that not all of their subjects behaved in the same fashion. Some subjects appear to be resistant to the effects of starvation in so far as their carbohydrate tolerance, urinary nitrogen excretion, blood ketone level, and serum lipids are concerned. If such persons are widely distributed in the population and could be screened in some simple preselection test, it might be of some value.

A series of experiments on the water requirements of castaways reported by Gamble (*Proc. Am. Philosophical Soc.* **88,** *151 (1944)*) confirms and extends the foregoing observations. These studies were directed toward finding the absolute minimum of renal water requirement and the conditions of the internal environment (food, water, and energy needs for activity) and of the external environment (radiant energy, air temperature, moisture, and wind velocity) which influenced water loss by renal and nonrenal channels. An interpretation of the data collected was made with a view to the necessity of severe restrictions on weight of water and food implicit in the use of lifeboats and inflated rafts.

By measuring the total output of solutes in the urine, the minimal water requirement for the subjects was found to be around 700 ml. per day when activity was low. When 100 g. of glucose were given in addition to 1200 ml. of water there was a large lowering of the minimal renal water need. "Both the antiketogenic and protein sparing effects of glucose reduce solute output, and thereby the renal water requirement, but only the protein sparing effect relates to the reduction of available body water." The reduction in water expenditure and therefore water need is approximately equal to the space required for the glucose. Perhaps even more important is the striking improvement in morale which occurs when glucose is taken rather than water alone.

Use of sea water with its higher salt content was shown to have objections which in the long run probably outweigh any slight gain in available water. The main trouble resides in a shuffling of electrolytes between sea water and body fluids giving rise to a reduction of the negative sodium balance and an increased deficit of potassium with ensuing changes of water distribution within the body. Practically, therefore, sea water does not help solve the problem.

Under conditions of stress (hot environments or hard work) the extrarenal water loss may dominate the picture. Expedients to conserve renal water may be wholly ineffectual when large quantities of water are lost from the lungs and skin to keep the body temperature regulated. Fortunately, simple procedures are usually available to castaways to reduce their external water loss. First, activity must be reduced to a mini-

mum. The others all have to do with avoiding the environmental factors which increase body heating, and seeking those which increase cooling. In other words provide shade, remove clothing, take periodic dips in the water or wet the body or clothing with sea water. Under the conditions of the experiments immersion of the body in water was more effective when done five minutes every half hour than when comfort alone was a guide. In this connection it might be pointed out that thirst is not a satisfactory guide to water requirement when the heat load is high (Bean and Eichna, *Federation Proceedings* **2,** *144 (1943)*), although in ordinary circumstances it may lead to a luxus consumption.

Much effort has gone into preventing or avoiding the disasters which may be followed by water deprivation and starvation, or finding temporary expedients to increase comfort and prolong the time of survival. The reports strongly indicate that a castaway will increase his chances of ultimate rescue by reducing or avoiding protein foods, by using carbohydrate for energy requirements, by avoiding any nonessential activity, and by keeping cool during a period when the supply of drinking water is restricted or nonexistent.

"FOLIC ACID" AND RELATED FACTORS

A few years ago Hogan and Parrott (*J. Biol. Chem.* **132,** *507 (1940)*) pointed out that under certain experimental dietary conditions chicks failed to grow and developed a severe anemia which could be cured with liver extracts. They suggested that an unrecognized factor of the B-complex was responsible, and for convenience, designated it as vitamin Bc. Campbell, Brown, and Emmett (*Ibid.* **152,** *483;* **154,** *721 (1944)*) have further described the physiologic activity of the substance which had been isolated in crystalline form from liver (Pfiffner *et al., Science* **97,** *404 (1943)*) and concluded to be vitamin Bc.

Chicks given a ration which produced (1) slow growth, (2) lack of feathering, (3) anemia, and (4) leucopenia, responded favorably, on all four counts, when given crystalline vitamin Bc. However, as might be expected, the quantitative amounts of Bc required for each type of response were different. Thus 100 micrograms of the vitamin per 100 g. of ration maintained growth over a four week period at the same levels as a good normal broiler ration, but only 40 micrograms were required to maintain normal values for the hematocrit, red blood cell count, hemoglobin, and thrombocytes. On the other hand, to prevent leucopenia, the optimum requirement was distinctly higher, being about 400 micrograms per 100 g. of ration. No differences in response were noted between oral and parenteral administration.

The Detroit group (Binkley *et al., Science* **100,** *36 (1944)*), has found that vitamin Bc exists in yeast as part of a more complicated molecule. Certain yeast extracts which are highly active in vitamin Bc as measured in the anemic chick have but little potency in stimulating the growth of either Lactobacillus casei or Streptococcus lactis. The method used for isolation of the active material from liver extracts was unsuccessful when applied to these yeast concentrates. However, upon enzymatic digestion the concentrate becomes highly active in microbiologic growth effects, and it becomes possible then to isolate a compound by the methods used with liver which corresponds in all chemical and biologic properties with the vitamin Bc previously prepared. These investigators have demonstrated that the chick antianemia activity in yeast extracts is due to the presence of vitamin Bc held almost entirely in the form of a relatively simple nonprotein conjugate. Nothing is

reported of the nature of the nonvitamin Bc portion of the conjugate.

The question naturally arises as to the relation of vitamin Bc and its conjugate(s) to certain recently studied unidentified nutritional factors which have similar chemical and physiologic properties, i.e. "folic acid," vitamins B_{10} and B_{11}, vitamin M, the S. lactis R factor of Keresztesy *et al.*, the new L. casei factor of Stokstad *et al.*, and others.

The term "folic acid" was first used by Mitchell, Snell, and Williams (*J. Am. Chem. Soc.* **63,** *2284 (1941)*) to designate a factor or factors found to be necessary for the growth of S. lactis R. Subsequent studies by many investigators using concentrates prepared in a similar manner from liver and other materials have shown that these preparations also contain biologic activity for the chick, rat, monkey, and other microorganisms. Therefore, responses elicited under these conditions have been often attributed to "folic acid." It has become evident that this term as now used does not refer to a single chemical entity. Some of the activity found in such preparations is due to vitamin Bc or its conjugates, while other effects may be due to parts of the vitamin Bc molecule or to entirely new substances.

The "folic acid" preparation of Mitchell, Snell, and Williams served as a growth factor for both S. lactis R and L. casei. Later, Keresztesy, Rickes, and Stokes (*Science* **97,** *465 (1943)*) isolated a growth factor for S. lactis R (SLR factor) which effectively replaced "folic acid" in the nutrition of this organism but was inactive for L. casei. Recently, Stokes, Keresztesy, and Foster (*Ibid.* **100,** *522 (1944)*) have shown that on incubation of their SLR factor with any one of a number of microorganisms including S. lactis R, a substance active for L. casei appears. The conversion is rapid and occurs in the presence of washed nongrowing cells. Unlike xanthopterin (*Nutrition Reviews* **2,** *54 (1944)*) SLR factor does not give rise to "folic acid" when incubated with rat liver suspension. Since the SLR conversion product supports growth for either S. lactis or L. casei, it would appear that S. lactis can use a more complex form of this factor than L. casei.

In contrast with the SLR factor, Hutchings, Stokstad, Bohonos, and Slobodkin (*Science* **99,** *371 (1944)*) have reported two crystalline preparations which are much more active for L. casei than for S. lactis R. Whether conversion is possible in these cases is not known.

After Wright and Welch (*Science* **98,** *179 (1943)*; *Nutrition Reviews* **2,** *54 (1944)*) showed that the incubation of liver suspension with xanthopterin led to an increase in L. casei and S. lactis growth stimulating effects ("folic acid"), xanthopterin was tested in a number of laboratories for its effect on experimental animals. Although Mallory, Mims, Totter, and Day (*J. Biol. Chem.* **156,** *317 (1944)*) reported a transient response in white blood cell count in both "sulfa" treated rats and vitamin M deficient monkeys, indicating that under some circumstances it may be active, all investigators, including Mallory *et al.*, now agree that xanthopterin has little or no activity for these animals. Likewise, xanthopterin has been found inactive for the chick. However, the pure compound has been shown to be effective in the prevention of an anemia which develops in fish reared on certain diets (Simmons and Norris, *Ibid.* **140,** *679 (1941)*).

Mallory *et al.*, working with yeast concentrates and an enzyme concentrate prepared from liver, have found that the leucopenia resulting from "sulfa" treatment of rats responds proportionally to the potential S. lactis content of the yeast, i.e. the S. lactis activity resulting from enzyme incubation. This unidentified factor could be the vitamin Bc conjugate of Binkley *et al.*, previously discussed.

Briggs, Luckey, Elvehjem, and Hart (*J. Biol. Chem.* **153,** *423 (1944)*) have reported the preparation of a series of concentrates from liver extract by varying methods and of different potencies in which the increased

growth response of L. casei and S. lactis does not parallel the response to growth and feathering in the chick. They also report a lack of correlation between feathering response and growth response in some of their preparations, and have therefore proposed the terms vitamin B_{10} and vitamin B_{11} to designate these supposedly new factors which they think are different from "folic acid" but probably closely related. It seems likely that these terms (B_{10} and B_{11}) will have but a temporary existence, since the possibilities are good that the responses noted will be explainable in terms of the crystalline preparation now available.

To summarize, the evidence seems to indicate that there are five distinct but interrelated factors involved in the biologic responses previously described, namely: (1) vitamin Bc, active for the chick, rat, S. lactis, L. casei, and probably also for the monkey; (2) Bc conjugate, active for the chick, and hence, possibly also for the rat and monkey but inactive for S. lactis or L. casei; (3) SLR factor, active only for S. lactis; (4) Stokstad L. casei factor, active only for L. casei; and (5) xanthopterin, active for fish, inactive for the rat and the two microorganisms, but convertible by rat liver into a substance active for L. casei and S. lactis. Inactivity of any of these substances for a given organism is presumably due either to (a) the presence of extra groups in the molecule which the organism is unable to remove, as seems to be the case with Bc conjugate, or (b) the absence of certain parts of the molecule which the particular organism cannot synthesize, which is perhaps the reason xanthopterin is inactive until incubated with rat liver.

An analogous situation obtains in regard to thiamine since certain microorganisms require only the pyrimidine portion of the molecule, and others require only the thiazole portion, whereas still other organisms, including man and rat, require the complete thiamine molecule. If microorganisms had been used in the early thiamine studies a number of different "factors" with thiamine activity would have been described. Confusion and duplication of terminology and lack of clear differentiation of independent factors are inevitable in the early stages of this type of investigation. However, the confusion is usually only temporary and rapidly resolves itself as pure compounds are isolated for testing. Investigations on this subject are very active, and within a few months many points of uncertainty should be resolved.

THE ANTIOXIDANT ACTIVITY OF GOSSYPOL AND COTTONSEED MEAL

Gossypol is the toxic principle of cottonseeds, which makes it impossible to use more than a small amount of otherwise suitable cottonseed meal in livestock rations. Recent evidence suggests however that in small proportions in the ration it may serve a valuable purpose. Some years ago Mattill (*J. Biol. Chem.* **90,** *141* (*1931*)) demonstrated that gossypol possesses antioxidant properties, being able to protect lard from oxidation *in vitro*. *In vitro* experiments by Hove and Hove (*Ibid.* **156,** *611, 623* (*1944*)) have extended our knowledge of the antioxidant activity of gossypol, by showing that it protects carotene dissolved in peroxidized ethyl oleate from oxidation. Its antioxidant activity in this reaction of carotene with peroxidized ethyl oleate was similar to that of the tocopherols.

In view of the known destruction of carotene which occurs in the intestine of the rat when linolate or linolenate is fed (Sherman, *J. Nutrition* **22,** *153* (*1941*)) and the effect of refined vegetable oils or alpha-tocopherol in preventing this destruction (Sherman, *Proc. Soc. Exp. Biol. Med.* **47,** *199* (*1941*); Quackenbush, Cox, and Steenbock, *J. Biol. Chem.* **145,** *169* (*1942*)), Hove has investi-

gated whether gossypol is effective in protecting carotene *in vivo*, and whether cottonseed meal can prevent carotene destruction and development of rancidity in ration components which carry fatty materials.

The animal experiments were made with rats which were taken from the breeding colony on reaching about 35 g. weight and given a basal diet very low in fat and all fat soluble factors except vitamin D. On this diet growth ceased after about five weeks and supplemental feeding was then started. If 2 micrograms of carotene were given to the rats daily, growth was regained for five or six weeks and then the rats declined and died within twelve weeks of the beginning of supplementation. In these rats xerophthalmia appearing during the initial period on the basal diet was cured in a week or two after they first received the carotene supplement, but symptoms of unsaturated fatty acid deficiency persisted and became more acute.

Rats which received a daily supplement of 2 micrograms of carotene plus 0.1 g. of lard had similar weight curves and lived about the same length of time, but their symptoms were of course different; they showed no signs of fatty acid deficiency but did continue to have xerophthalmia and developed severe incoordination and leg weakness. A daily supplement of 2 micrograms of carotene plus 0.8 mg. of alpha-tocopherol caused growth to continue for eight weeks, after which weight was maintained; all signs of vitamin A deficiency disappeared, but scaliness around the paws and nose due to fatty acid deficiency persisted. If both lard and tocopherol were given in addition to the carotene, good growth occurred throughout the experimental period and no signs of deficiency appeared at all. Gossypol was tested by comparing the protective activity of equal doses of gossypol and alpha-tocopherol. These were found to be similar, the gossypol being slightly inferior in protective power.

For the *in vitro* experiments, carotene was dissolved in ethyl oleate and added to several common constituents of livestock feeds which had been finely ground and passed through a 24 mesh screen, so that each contained 80 p.p.m. of carotene and 30 per cent of ethyl oleate. The different materials were then stored at 58° in open dishes, and 2 g. samples were taken for carotene determination at the beginning of storage, and also after six, twenty, and thirty-one days. The original tocopherol content was also measured. The only material which protected the carotene for over thirty-one days as well as cottonseed meal was wheat germ; the two showed equal protective activity. Further experiments were made to determine by taste test the length of time taken for rancidity to develop in cottonseed, soybean, and peanut meals. It was observed that the addition of 10 per cent of defatted ground cottonseeds to peanut meal caused the peanut oil to keep four to five times as long as it would without such addition, while the addition of 10 per cent of cottonseed meal increased the keeping time between 60 and 70 per cent.

The tocopherol content of the cottonseed products was much too low to account for their protective activity for carotene, or for the prevention of rancidity in peanut meal, and Hove considers that this activity may be related to the gossypol in the cottonseed. He points out that 1 mg. of gossypol fed daily to his test rats would correspond to approximately 10 per cent of cottonseed meal in the ration, which is a safe level to feed to most farm animals. It would appear therefore that this amount of cottonseed meal in animal rations could bring about a more efficient preservation and utilization of carotene in feedstuffs, which would be a highly desirable result during the present period of short supply of vitamin A for stock feeding. Furthermore, if by suitable chemical treatment the gossypol could be rendered less toxic while retaining its full antioxidant activity, much greater benefits could be obtained from the use of cottonseed products

in animal rations. Hove demonstrated that, molecule for molecule, dianilido-gossypol is equally as effective as gossypol in protecting carotene, but no data on the comparative toxicity of the two compounds are yet available.

LABORATORY RECOGNITION OF VITAMIN DEFICIENCIES

Much has been written regarding the use and abuse of vitamins in the treatment of mild and early deficiency states (see *Nutrition Reviews* **2**, *353* (*1944*)). Every worker in this field has recognized the need for the development of objective methods for the measurement of vitamin deficiencies. From this viewpoint, data presented by Ruffin, Cayer, and Perlzweig (*Gastroenterology* **3**, *340* (*1944*)) deserve scrutiny.

Two groups of patients and a series of 7 to 100 normal controls were studied. The patients included one group of 17 to 26 cases of vitamin deficiency as indicated by the presence of such symptoms as glossitis, papillary atrophy of the tongue, cheilosis or peripheral neuritis, together with a history of inadequate diet. No reliance was placed on such symptoms as weakness, nervousness, anorexia, fatigability, vague digestive disturbances, or vascularization of the cornea. A second group of 9 to 16 patients ("ward controls") gave no definite evidence of a deficiency state as judged by the criteria mentioned above. Their usual diet was that of the average tenant farmer.

As might be expected the average values for the various determinations were highest in the normal controls, intermediate in value in the "ward controls," and lowest in the cases with obvious symptoms of vitamin deficiency. Study of the findings in the individual cases, however, shows that some of the values tended to overlap considerably. Thus the plasma vitamin A level showed extreme ranges which were almost identical in all the groups. The plasma vitamin C levels ranged as low as 0 even in the normal controls. On the other hand there was a striking difference between the controls and the cases of deficiency in respect to urinary excretion of N-methylnicotinamide after the oral administration of 500 mg. of nicotinic acid amide. Less sharp but nevertheless substantial were the differences in the levels of excretion of riboflavin following a test dose, and those for thiamine following the administration of 1 mg. intramuscularly. The levels for pyridoxine showed no significant differences between the normal controls and the vitamin deficiency group.

Instead of using as the standard for comparison the lowest value for each vitamin as found in the control group, it was decided arbitrarily to select that level which was at the upper limit of the lowest 10 per cent of the normal control group. This was designated as the "suggested lower limit of normal" and was done only to facilitate comparison of data. In the case of vitamin C the "suggested lower limit of normal" was 0.15 mg.; for vitamin A it was 75 I.U. per 100 ml.; for niacin 65 mg. excreted in fourteen hours; for riboflavin 2200 micrograms excreted in twenty-four hours following oral administration of 5 mg.; for thiamine 300 micrograms excreted in four hours following the intramuscular injection of 1 mg. The observations showed that 24 of the 26 patients who had clinical evidence of a deficiency state based upon the clinical criteria enumerated had levels below the suggested lower limit of normal for nicotinic acid, riboflavin, and thiamine. The laboratory data indicated the prevalence of multiple deficiencies.

The authors make no claim to have given the final answer to the laboratory diagnosis of vitamin deficiencies but their serious attempt at objectivity gives hope that in the course of time such laboratory procedures may help to differentiate between the constitutionally inadequate individual and the patient who has a true vitamin deficiency.

THE EFFECT OF VITAMIN A DEFICIENCY ON THE SKIN

In their classic report Wolbach and Howe (*J. Exp. Med.* **42,** *753* (*1925*)) defined the lesion of vitamin A deficiency as atrophy of many glands, arrest of growth, emaciation and replacement of many different single-layered epitheliums by stratified keratinizing epithelium. This process of change from cuboidal or columnar epithelium to the stratified type is known as keratinizing metaplasia and has been demonstrated as occurring in the eye, the paraocular glands, and the respiratory, gastrointestinal, and genitourinary systems. Frazier and Hu (*Arch. Int. Med.* **48,** *507* (*1931*)) described a dermatosis in Chinese soldiers which they termed follicular hyperkeratosis. In the majority of their patients there were ocular signs of vitamin A deficiency, and the diets of the soldiers were deficient in vitamin A. The skin lesion consisted of hyperkeratosis and atrophy of hair follicles followed by plugging and hypertrophy of hair follicle epithelium. As Sullivan and Evans (*Arch. Dermat. Syph.* **51,** *17* (*1945*)) point out, all discussions of vitamin A deficiency since Frazier and Hu's report include summaries of the internal changes demonstrated in animals and the cutaneous alterations described in man. There has developed an acceptance of the concept that the cutaneous lesion due to vitamin A deficiency is similar to that of other epithelium and that it is the result of so-called keratinizing metaplasia.

Sullivan and Evans indicate, however, that on histologic grounds this concept is difficult to accept. In the skin the epithelium is of the stratified keratinized type. Therefore, keratinizing metaplasia in its literal meaning is an impossibility unless atrophy, which precedes the "metaplasia," is so profound that a single-layered epithelium is produced. They call attention to the fact that in the earlier experimental studies of vitamin A deficiency, the deficiencies which existed were complex and included deficiencies of the vitamin B-complex, fat, and essential fatty acids, as well as of vitamin A. When young rats were fed a diet deficient only in vitamin A, Sullivan and Evans (*J. Nutrition* **25,** *319* (*1942*)) observed a deficiency disease the gross and microscopic signs of which differed from the previously recorded descriptions of vitamin A deficiency. In such animals there were no gross cutaneous alterations except during the very late stages of the deficiency, when it was not possible to exclude the effects of complicating deficiencies and the nonspecific effect of deficiency. The histologic changes differed from those described by Frazier and Hu. Their observations were confirmed by the report of Moult (*Arch. Dermat. Syph.* **47,** *768* (*1943*)).

Studies by Sullivan and Evans in rats have suggested that the signs of vitamin A deficiency may be delayed by increasing the amount of vitamin B-complex in the vitamin A deficient rat and that the deficiency signs may be precipitated by inadequacy of vitamin B-complex and fat in a vitamin A deficient diet. Such observations indicate an interrelationship of vitamin A and the vitamin B-complex and led to their suspicion that vitamin B-complex deficiency preceded or accompanied vitamin A deficiency in man when follicular hyperkeratosis such as that described by Frazier and Hu was present. Although in Frazier's opinion his subjects partook of diets adequate in B-complex and fat, a report by Reiss (*Chinese Med. J.* **50,** *945* (*1936*)) described similar if not identical cutaneous lesions in natives subsisting on diets known to be deficient in the vitamin B-complex as well as in vitamin A. These considerations led Sullivan and Evans to plan experiments with the object of determining the effect of superimposing vitamin A deficiency on vitamin B-complex deficiency (other than thiamine). In this way an opportunity could be provided for evaluating experimentally the effect of vitamin A deficiency on severely atrophied cutaneous

epithelium. The conditions of such an experiment should be ideal for producing keratinizing metaplasia of the skin if such a phenomenon is possible.

These workers found that rats given a diet devoid of vitamin B-complex other than thiamine developed atrophic changes in the skin and appendages. The vitamin B-complex deficiency was continued until the animals were practically moribund. Vitamin A was then withdrawn and the vitamin B-complex was added in an amount barely sufficient to maintain life. Thus vitamin A deficiency was produced in sick rats with atrophic single-layered cutaneous epithelium and atrophic, disintegrated hair follicles and sebaceous glands. Microscopic cutaneous alterations developed in the skin consisting of only a partial restitution of the epithelium, and excessive keratinization occurred in the widely dilated and atrophied follicles and in the wavy epidermis. There was no hypertrophy of portions of the hair follicle epithelium, however, such as Frazier and Hu reported in Chinese soldiers. The collections of excessive hyperkeratinization accumulated to form small squamous plaques rather than horny spicules. There were no mounds of epidermis with craters from which the horns projected, this being probably due to lack of proliferation of hair follicle epithelium. It is pointed out that on casual glance there may be some resemblance of the lesion of the rat to the conventional notion of the gross lesion in the human adult, but a critical appraisal of the gross changes viewed in conjunction with the microscopic changes demonstrates dissimilar features of this and the original lesion of Frazier and Hu.

The study by Sullivan and Evans demonstrates once again the confusion which has unfortunately crept into the vitamin literature. Their studies apparently reveal an example of the error introduced by substituting the findings in one organ, the skin, of one species, man, in the scheme of findings in organs other than the skin for another species, namely, the rat. As has been demonstrated several times before, such substitutions cannot always be made.

THE NUTRITIVE VALUE OF HUMAN PLASMA

The biologic value of various types of protein has been the subject of many investigations. The value of different proteins for regeneration of plasma or hemoglobin has been studied at some length (Madden and Whipple, *Physiol. Rev.* **20,** *194* (*1940*)). Plasma protein itself was found to be one of the most potent agents in the regeneration of plasma protein when fed to dogs depleted by plasmapheresis. Orten and Orten, however, found that beef serum was not as effective as lactalbumin in supporting growth of rats (*Federation Proceedings* **2,** *67* (*1943*)). These opposed findings in the dog and rat led Hegsted, McKibbin, and Stare (*J. Clin. Invest.* **23,** *705* (*1944*)) to reinvestigate the problem by feeding human plasma protein to rats. The question is worthy of consideration because of the use of human plasma proteins and various fractions and concentrates as the main or only source of protein when certain patients are maintained by parenteral feedings alone.

Young male weanling rats weighing 30 to 35 g. were placed in groups of 3 on a purified ration which varied only in the source of protein—dried plasma, casein, zein or gelatin singly or in combinations. It was found that plasma alone supported meager growth when compared with casein. The amino acid content of casein differs from that of plasma proteins chiefly in the higher concentration of isoleucine. When a supplement of 0.5 per cent isoleucine was added to plasma proteins the growth response was equivalent to that which occurred when

casein was fed as the sole source of protein. Although only a small number of animals was used the results were consistent and appear significant.

These experiments have been extended by additional studies on the nutritive value of human plasma protein fractions for dogs as well as for rats reported by Hegsted, Hay, and Stare (*J. Clin. Invest.* (*in press*)). In the rat studies plasma fractions amounting to 20 per cent replaced sucrose in the basal ration (4 per cent salt mixture, 4 per cent corn oil, and 92 per cent sucrose). Vitamins were added in excess of requirement. Groups of 3 rats with average weight of 52 g. were given human gamma globulin, fibrin, or albumin as the only protein. Control rats had skim milk powder as the source of protein. Fibrin supported growth as well as that occurring with skim milk, albumin supported no growth, while globulin was intermediate in its capacity to support growth.

In another series of tests four groups of young rats were fed rations containing 20 per cent albumin (1) unsupplemented; (2) with 0.3 per cent l(+)-tryptophane; (3) with 1.2 per cent dl-isoleucine; and (4) with 0.3 per cent l(+)-tryptophane plus 1.2 per cent dl-isoleucine. Growth was rapid in group (4). It occurred in the other groups only after the tenth day when additional supplements were added so that both amino acids were given. An effort was made to find the approximate amounts of these amino acids which would give optimum growth in young rats with 20 per cent protein in the ration. It was found that if dl-isoleucine was provided at constant levels in excess of requirement, a total of 0.085 per cent l(+)-tryptophane gave results as good or better than with higher levels. A protein fed at a 20 per cent level would have to contain 0.42 per cent l(+)-tryptophane for best results.

In a similar experiment dl-isoleucine was fed at various levels while l(+)-tryptophane was given in constant amounts in excess of needs. Maximum growth was obtained when approximately 0.9 per cent dl-isoleucine was added. Calculations of the dl-isoleucine in the diet indicated that optimum growth occurred when it constituted 0.78 per cent of the diet or 3.9 per cent of the protein in the diet.

Further studies of a similar kind were carried out on the adult dog to determine the minimum amount of protein required to maintain nitrogen equilibrium during six day periods following ten days on a "nitrogen free" basal ration. When albumin was the sole source of nitrogen, approximately 0.14 g. of nitrogen per kilogram per day was required for positive balance. This is equivalent to a protein requirement of 0.875 g. per kilogram.

A comparison showed that the requirements of the rat and dog for specific amino acids under the circumstances of the study differ more than threefold. It emphasized the variation in requirement for essential nutrients in different species. While it is not permissible to make a strict comparison between protein requirement for nitrogen balance in adult dogs and for growth in immature rats the data are in keeping with the fundamental fact of species differences in the requirement of various essential nutrients.

It must therefore be emphasized, as these investigations make particularly clear, that these findings cannot be translated into requirement estimates for human subjects when their sole source of protein comes from albumin or plasma administered intravenously. They must be extended by studies of human intravenous feedings. It has been reported that isoleucine is an essential amino acid for the human adult (Rose, Haines, Johnson, and Warner, *J. Biol. Chem.* **148,** *457 (1943)*). It is probable that isoleucine has other functions than as a building block in compounding plasma proteins. It may be stored in other tissues than plasma and certainly is utilized in the formation of many body proteins. If such be the case it will be necessary to revise the current concept of

plasma proteins as a readily available storehouse for the essential amino acids in proportions representing the total stored amino acids.

NUTRITION OF INSECTS

From a strictly academic point of view the subject of the nutritional requirements of insects offers a vast and almost virgin field of study. A few insect species have been used as experimental animals in dietary investigations, and the results have been summarized by Craig and Hoskins (*Ann. Rev. Biochem.* **9,** *617* (*1940*)) and by Trager (*Physiol. Rev.* **21,** *1* (*1941*)). But the requirements of most of the 500,000 species of insects are completely unknown and even those that have been studied involve only a very small number of experiments in comparison with the many that have been devoted to the rat, dog, guinea pig, or chick. Nevertheless, insects possess certain advantages as nutritional test objects. They are cheap, abundant, and easy to raise in large numbers. They require only small amounts of food, and many experiments can be performed with comparatively simple equipment. Thus, great economy of space, time, and material can be effected. Furthermore, in the phylogenetic scale, insects lie between the microorganisms, many of which have been used in vitamin assays, and the higher animals. They therefore possess many of the biochemical characteristics of both the higher and the lower forms of life and it is these similarities that give value to studies on insect nutrition.

For example, insects appear to be like higher animals in their requirements for better known members of the vitamin B-complex. It has been found that the fruit fly (Drosophila melanogaster) requires thiamine, riboflavin, niacin, pyridoxine, and pantothenic acid (Tatum, *Proc. Nat. Acad. Sci.* **27,** *193* (*1941*)); the mosquito larva (Aedes aegypti) also requires the same vitamins (SubbaRow and Trager, *J. Gen. Physiol.* **23,** *561* (*1940*)). Golberg, DeMeillon, and Lavoipierre (*Nature* **154,** *608* (*1944*)) have found that mosquitos need "folic acid" for pupation, and that biotin is an essential growth factor. Fraenkel and Blewett (*Biochem. J.* **37,** *686* (*1943*)) have studied the nutritional requirements of the larvae of 5 beetles, Tribolium confusum (flour beetle), Lasiderma serricorne, Sitodrepa panicea, Silvanus surinamensis, and Ptinus tectus, by growing them on synthetic diets containing all the chemically identified members of the vitamin B-complex. These workers found that thiamine, riboflavin, niacin, pyridoxine, and pantothenic acid are essential for the life of Tribolium and Ptinus, while choline and biotin may be of great importance as growth factors. The other 3 beetles do not seem to require some of the vitamins which are indispensable for Tribolium and Ptinus. This difference has been found to be due to the presence of intracellular symbionts which synthesize some of the vitamins. The same authors also have observed (*Ibid.* **37,** *692* (*1943*)) that certain insects require sterols in the diet. Any one of several common plant or animal sterols met this requirement, but interestingly enough calciferol (vitamin D_2) was not utilized by any of the insects tested.

At the Nutrition Research Laboratories of Coonoor, India, considerable attention has been given to the nutritional requirements of the rice moth larvae (Corcyra cephalonica St). The technic employed in these studies was similar in principle to that used ordinarily in studies with rats or other higher animals. A stock colony of larvae, pupae, and moths was maintained on a whole wheat diet in an incubator kept at a temperature of 30°C. and a humidity

of 75 per cent. After hatching, the larvae were kept on a diet of whole wheat flour for ten days prior to transfer on experimental diets. They were then weighed every week and their growth studied. It was shown that the rice moth larva grows poorly or not at all unless a fat soluble factor, sterol, is fed (Sarma and Sreenivasaya, *Current Sci.* **8,** *551* (*1939*); **10,** *525* (*1941*)), and also several members of the vitamin B-complex: thiamine, riboflavin, and pyridoxine (Sarma, Swamy, and Sreenivasaya, *Ibid.* **11,** *332* (*1942*); Sarma, *Indian. J. Med. Res.* **31,** *165* (*1943*)). Attempts were next made to assay foodstuffs for vitamins by employing the vitamin-deficient larva as the experimental animal. The values obtained for the pyridoxine content of wheat, rice, milk, and meat agreed fairly well with the results of chemical analyses or bioassays with higher animals. It is pointed out, however, that different strains of the same insect species may show quantitative differences in their requirements for a given vitamin, and hence before routine assays are performed with them, pure strains of insects would be highly desirable.

The results obtained with insects are also interesting from a physiologic and metabolic point of view. It was observed, for instance, that on a thiamine-deficient diet the rice moth larvae accumulated up to eight times as much pyruvic acid as was present in the normal organism (Bhagvat and Sarma, *Indian J. Med. Res.* **31,** *173* (*1943*)). The pyruvic acid content of the deficient larvae decreased rapidly when they were transferred to a diet rich in thiamine. Histologically the thiamine-deficient larvae showed characteristic degeneration of nerves and fatty tissues (Swamy and Sreenivasaya, *Current Sci.* **11,** *147* (*1942*)). Like the rat, the rice moth larvae appeared to synthesize ascorbic acid; at least, no external source of this vitamin was required for growth (Sarma and Bhagvat, *Ibid.* **11,** *394* (*1942*)). Further, a yellow compound was excreted by the rice moth larvae when they were fed a pyridoxine-deficient diet containing additional tryptophane, and the yellow substance disappeared from the excreta when pyridoxine was included in the diet. Of the several amino acids examined, only tryptophane gave rise to the yellow colored excreta.

The science of nutrition, as McCay has emphasized (*Science* **86,** *347* (*1937*); *J. Am. Med. Assn.* **110,** *1441* (*1938*)), should rest on a knowledge of the nutritional needs of many different types of organisms. Elvehjem has also recently laid stress on the continued use of many kinds of living organisms and of manifold approaches to nutritional problems (*Nutrition Reviews* **2,** *33* (*1944*)). The ultimate objective of promoting the welfare of man will not be attained by studying man alone. Much of our knowledge of the vitamins and of their mode of action depends upon investigations of enzyme systems and the nutritional requirements of microorganisms. Studies of the factors required for the growth of insects therefore promise to be of value in our understanding of the physiology of organisms in general.

MANGANESE IN BONE DEVELOPMENT

Careful analysis of the evidence concerning the essentiality of manganese in nutrition (*Nutrition Reviews* **1,** *394* (*1943*)) leads to the conclusion that this element may now be considered necessary for normal growth, reproduction, lactation, and bone formation and that it plays a role in certain enzyme systems. In so far as bone formation is concerned, the need for manganese has been demonstrated in the chick (Wilgus, Norris, and Heuser, *J. Nutrition* **14,** *155* (*1937*)), in the pig (Miller, Keith, McCarty, and

Thorp, *Proc. Soc. Exp. Biol. Med.* **45,** *50 (1940)*), and in the rat (Wachtel, Elvehjem, and Hart, *Am. J. Physiol.* **140,** *72 (1943)*).

Nevertheless there have been contradictions in the literature concerning the role of manganese, and it appears that other factors in the diet may influence the availability of manganese or change the picture of bone perosis. Thus, Jukes (*J. Biol. Chem.* **134,** *789 (1940)*) showed that the presence of choline, in addition to manganese, is necessary for complete protection against perosis. Also Wilgus and Patton (*J. Nutrition* **18,** *35 (1939)*) noted that increased amounts of calcium and phosphorus in the diet intensify the symptoms of manganese deficiency.

Smith, Medlicott, and Ellis (*Arch. Biochem.* **4,** *281 (1944)*) now report that in the rabbit a manganese deficiency also seriously interferes with normal bone development. When young rabbits were fed whole milk powder ad libitum, or cow's milk enriched with 10 per cent of whole milk powder, their front legs developed serious deformities. This condition was prevented by a daily supplement of 2 to 5 mg. of manganese. The growth of those rabbits not receiving the manganese supplement was significantly less than that of positive control animals. The weight, density, length, breaking strength, and ash of the bones were all less in the manganese deficient rabbits. The reduction in the percentage of bone ash was much less than that which occurs in rickets; also the bones differed histologically from rachitic bones, the most evident change being a narrowing of the zone of provisional calcification owing to an appreciable reduction in the number of cartilage spicules. A suppression of osteogenesis was suggested.

It seems probable that extra calcium and phosphorus in the diet renders dietary manganese less available, either by interfering with its absorption or by removal of manganese ions from solution during digestion. Under such conditions, normally adequate amounts of manganese may be insufficient for proper bone development. A realization of the essential nature of manganese has been recognized in practical animal feeding. Most poultry feeds and proprietary mineral mixtures, as well as some other livestock feeds, now contain supplementary manganese in addition to calcium and phosphorus.

SERUM ALBUMIN REGENERATION BY PROTEIN HYDROLYSATES

The steps by which protein is digested in the alimentary canal, absorbed, and reconverted into plasma and general body protein have been the subject of numerous experiments in recent years. Knowledge of the process whereby this is accomplished is valuable not only for the understanding it gives of fundamental biologic processes but because it has resulted in the development of valuable technics in parenteral therapy which may save lives. Because of the potential difficulties in protracted or repeated intravenous injections it is important to determine whether protein hydrolysates are used more effectively when given parenterally than when taken into the alimentary canal by mouth or by stomach tube. Such a study on normal dogs was undertaken with casein, lactalbumin, and beef serum protein and their hydrolysates by Cox and Mueller (*J. Clin. Invest.* **23,** *875 (1944)*). Preliminary experiments on the weight gain of rats were made with some of the materials.

It is well known that ingested proteins vary considerably in their ability to cause regeneration of plasma albumin. Cox and Mueller found that the hydrolysates of casein, lactalbumin, and beef serum protein did not differ significantly in their capacity to induce regeneration of serum albumin in dogs when given by vein. Assay values

for the same hydrolysates given orally were substantially the same as when the intravenous route was used except in the case of beef serum protein in which the orally administered material seemed to effect about twice the regeneration of serum albumin as did that given intravenously. This point requires further study on a large number of animals.

Since the hydrolysates of several different proteins appear to give rise to approximately equivalent regeneration of serum albumin while the intact proteins fed by mouth differ markedly in the resulting regeneration, it appears that different rates of hydrolysis of protein in the gut, or different rates of absorption of the amino acid end products of such hydrolysis, must prevail. When hydrolysates rather than intact proteins are fed, the variable of differing rates of amino acid liberation is excluded and when the hydrolysates are injected intravenously the variable of differing rates of absorption is excluded. Since the experiments cited indicate that plasma albumin regeneration is equally good whether hydrolysates are given orally or parenterally, it appears that the rate of intestinal hydrolysis of proteins is the factor responsible for the differences in the ability of various types of intact protein of good nutritive quality to regenerate plasma albumin in dogs. If these findings should prove to apply in man, the rationale of oral rather than intravenous administration of protein hydrolysates would facilitate the use of such materials in the many instances where the parenteral route is not satisfactory.

WHEAT GLUTEN AND CANINE HYSTERIA

Canine hysteria is an ill defined malady whose nomenclature, origin, and treatment are still in dispute. Other terms that have been suggested for this disease, and used by veterinarians, are "running fits," "fright disease," "epizootic hysteria," "enzootic hysteria," "infectious hysteria," and "hyperkinesia." The symptoms generally associated with the disease are paroxysmal attacks of hyperexcitability characterized by running, barking, or howling, faulty vision, deranged balance, dementia, and often clonic convulsions. A heavy mucous saliva may be secreted during the convulsions, and urination and defecation occur frequently; the affected dogs seem indifferent to injury or to efforts to quiet them. The onset and frequency of attacks are reported to be influenced by excitement, physical strain, and heredity. The subject has been reviewed by Hewetson (*Vet. Record* **48,** *1202 (1936)*). Between attacks the animal may appear normal except that it is often nervous and apprehensive. Attacks are usually precipitated by stimuli such as a sudden noise or a flash of light. Other attacks, apparently set off by internal stimuli, arouse sleeping or resting animals.

Of particular interest to nutritionists is the fact that canine hysteria can be both produced and cured by diet. Melnick and Cowgill (*J. Nutrition* **14,** *401 (1937)*) observed hysteria among experimental dogs receiving rations containing gliadin. Arnold and Elvehjem (*J. Am. Vet. Med. Assn.* **95,** *303 (1939)*) produced canine hysteria by feeding a baked diet of meat scrap and wheat flour; supplements of casein, dried canned dog food, and lysine appeared to be helpful in preventing attacks. Morgan and Groody (*J. Am. Vet. Med. Assn.* **105,** *406 (1944)*) reported a decrease in the frequency of attacks when casein was added to their hysteria producing ration. However, both of these latter groups of workers recognized that a toxicity might also be involved.

Patton (*Vet. Med.* **34,** *372 (1939)*) fed a commercial dry dog ration and observed many of the characteristic symptoms of canine hysteria in adult dogs within ten to

twelve days. When the ration was autoclaved and fed to young, growing dogs, symptoms appeared within three to four days. Shortly after the onset of the attacks vitamin B_1 was supplied as tikitiki and as the crystalline vitamin, and many of the symptoms disappeared. From these observations the author concluded that canine hysteria was due to a thiamine deficiency. It would appear doubtful, however, whether an uncomplicated acute thiamine deficiency could arise in such a short time, and a characteristic of canine hysteria is that the animals frequently regain a normal appearance between attacks even in the absence of curative measures.

It has frequently been noted that canine hysteria is associated with baked dog foods. In a recent study Wagner and Elvehjem (*J. Nutrition* **28,** *431 (1944)*) compared the hysteria-producing activity of nine commercial dog foods and found that four samples were active in varying degrees, while five showed no activity. In further experiments with the active samples, supplements of thiamine hydrochloride, casein, dried canned dog food, and lysine dihydrochloride failed to prevent attacks of hysteria. However, some increase in growth resulted from the addition of the lysine, casein, and dried canned dog food to the ration.

About the same time and in the same laboratory Parry (see Wagner and Elvehjem, *J. Nutrition* **28,** *431 (1944)*) noted that young dogs fed commercially produced wheat gluten developed all of the usual symptoms of canine hysteria and in high degree. Accordingly diets high in wheat gluten were used routinely to produce symptoms of hysteria. In the first experiments 30 per cent of wheat gluten replaced the 27.5 per cent of casein normally fed, and symptoms of hysteria developed within three to four days. Attempts to prevent the symptoms by adding other essential nutrients failed. The attempts included 20 per cent of casein, 18 per cent of gelatin, 18 per cent of casein hydrolysate, 4.97 per cent of dl-lysine monohydrochloride, and higher levels of thiamine and pyridoxine.

The severity of the symptoms and the rapidity of their development appeared to depend upon the level of wheat gluten in the ration. When 10 per cent of wheat gluten was incorporated into a ration containing 20 per cent of casein, the symptoms developed within twenty-two days; when 25 per cent of wheat gluten was fed in this ration, symptoms developed within seven days. Intermediate levels of wheat gluten produced intermediate effects. Sensitivity to wheat gluten appeared to be characteristic only of the dog; diets high in wheat gluten were fed for many weeks to the cat, rat, mouse, chick, guinea pig, and monkey without any symptoms of toxicity.

From these studies Wagner and Elvehjem concluded that canine hysteria is dietary in origin. The possibility of an infectious agent appeared remote because normal dogs and those affected with hysteria were often in close association, usually separated only by a wire mesh. Attempts to produce toxins from organisms isolated from the gluten gave negative results, and furthermore the hysteria producing activity of the wheat gluten still persisted after six hours of autoclaving. The disease was considered to be the result of a toxicity rather than that of a deficiency because the search for protective factors was so consistently unsuccessful. The nature of the toxic principle, however, has not been determined, although selenium does not appear to be involved. The wheat gluten was analyzed for selenium because this element is known to accumulate in certain samples of toxic wheat, but the samples fed by Wagner and Elvehjem were uniformly low in this element. It did not seem probable that the gluten disturbed gastrointestinal functions since it readily became water soluble by digestion with pancreatin.

It would be highly desirable to know whether grains other than wheat may also give rise to the symptoms of canine hysteria.

There is also the possibility that different samples of wheat used in the manufacture of commercial dog foods differ in their content of gluten. Furthermore, one would like to know to what extent commercial processes contribute to the elaboration of the toxic principle. There is some evidence that lower levels of gluten can produce hysteria if fed for very long periods of time; characteristic symptoms have been observed occasionally in dogs fed bread and milk for many months. It is also possible that the occasional fits observed in dogs in rural communities may be due to the common practice of feeding large quantities of bread to the family dog.

ANEMIA IN ADULTS WITH SCURVY

There has been considerable discussion in medical papers on scurvy concerning the nature of the anemia which sometimes accompanies the other manifestations of classic ascorbic acid deficiency in man. After considering the available evidence, Ralli and Sherry (*Medicine* **20,** *251* (*1941*)) concluded that the absence of ascorbic acid could not be considered solely responsible for the anemia that may be present in clinical scurvy, at least in human infants and adults.

The results of a study of 53 cases of scurvy in adults over 40 years of age reported by McMillan and Inglis (*Brit. Med. J.* **2,** *233* (*1944*)) have been reviewed earlier (*Nutrition Reviews* **3,** *24* (*1945*)). It was not possible to evaluate critically the role of ascorbic acid, iron, and other factors in producing the anemia.

New evidence on the subject has been presented by Vilter and Woolford (*Proc. Central Soc. Clin. Res.* **17,** *34* (*1944*)). They found a definite anemia in 8 of 10 adults with scurvy observed recently in the Cincinnati General Hospital. Reticulocytosis ranging from 3 to 10 per cent (except for 2 patients in whom it was only 1 per cent) and moderate hemolytic jaundice led these observers to believe that hemolysis was at least one of the causative factors.

Red blood cell counts ranged from 1.74 to 3 million per c.mm. and hemoglobin levels from 5.8 to 10.5 g. per 100 ml. Cells were normocytic, normochromic, or moderately macrocytic hyperchromic. Half of the cases had white blood cell counts below 6000 though the differential counts were normal in all cases. Platelets were normal.

Sternal aspiration yielded bone marrow which was hypocellular, though there was a relative increase in the number of erythrocyte progenitors, the majority of which were at the normoblastic or late erythromblastic stages of maturation. Cells of the granulocyte series appeared to be normal.

The icterus index was found to range from 10 to 22; there was an excess of urobilinogen but no bile in the urine. In every case the indirect Van den Berg reaction was obtained on the blood serum. It was found that there was no correlation between the extent of ecchymoses and the degree of jaundice or the severity of the anemia. It is of interest that no new ecchymoses appeared after the patients were hospitalized.

As a control the patients were fed a diet which did not contain any ascorbic acid and which was low in the B-complex vitamins. Erythrocyte counts and hemoglobin levels remained stable in 6 patients and hemoglobin levels declined in 2 in spite of high or slowly rising erythrocyte counts. The control regimen was followed for two to fourteen days until mental torpor and impending vascular collapse made imperative the administration of ascorbic acid which was given at a level of 500 mg. daily, presumably by mouth. Following the institution of this therapy, while the same basic diet was given, one patient showed a reticulocyte rise from 5 to a maximum of 10 per cent (on the sixth day) and in another there was an increase

from 2 per cent to 19 per cent on the tenth day. In the other 6 patients the initially high reticulocyte counts were maintained for six to ten days. Increases in red blood cells and hemoglobin values began one week after therapy with ascorbic acid was begun, and by the end of three weeks the levels for erythrocytes reached or surpassed 3.5 million per c.mm. and 12.5 g. of hemoglobin per 100 ml. in all patients. Icterus indices returned to normal when the red cells began to rise and the bone marrow became normal in those in whom it was studied.

Though these results have been reported in abstract form only, the careful controls and the extensive studies carried out are impressive and it is hoped that publication of the detailed report will finally permit the unequivocal settlement of the heretofore rather vaguely approached problem of the "anemia of scurvy."

NUTRITION AND INTESTINAL PARASITES

Although there is considerable evidence to indicate that poor nutrition is an important predisposing factor to hookworm and related parasitic infestations in man and experimental animals (*Bull. National Research Council no.* **109** (*1943*)), more complete, specific, and quantitative information on this point is sorely needed. With man, the evidence consists mainly in the observation that those areas of the world where the greatest infestation occurs are almost without exception areas where malnourishment is highly prevalent, and that those individuals in such areas who are so affected are the ones who are the least well nourished. It is the contention of many investigators, based on small-scale studies, that injury to the host by the worm (i.e., anemia) may not only be reduced, but in many cases the infestation can be removed by better diet alone. From numerous animal experiments it seems well established that diet can be an important factor affecting the reaction of the host to parasitic infestation. However, the specific dietary factors involved have never been clearly determined. Watt (*Am. J. Hygiene* **39,** *145* (*1944*); Watt, Golden, Olason, and Mladinich, *Science* **97,** *381* (*1943*)) published evidence which indicates that this dietary effect is probably nonspecific in character, i.e., any one of a number of deficiencies may serve as a predisposing factor. This is of interest because it serves as another example of the importance of general good nutrition for body resistance. It also emphasizes the fact that good nutrition is important for reasons other than the prevention of deficiencies themselves.

Watt has compared the resistance of groups of normal and deficient rats to infection with the roundworm, Nippostrongylus muris. Experimental deficiencies in vitamin A, thiamine, or riboflavin were produced. Four criteria of resistance were used: (1) effects of deficiency upon resistance to primary infection; (2) effects of deficiency upon the resistance to superinfection; (3) influence of deficiency upon the resistance to subsequent reinfection; (4) effects of deficiency upon the capacity of the animal to form immune bodies as demonstrated by passive transfer. Adequate, well nourished control series were used for comparison in each set of experiments.

The first type of experiment (primary infection) was carried out as follows: Soon after the animals on the various deficient diets began to show a decreased growth rate, both they and the controls (on normal diets) were fed a standard number of larvae (2300 to 2500). After twelve days the number of worms found in the intestine at autopsy were counted. With all three deficiencies the number of worms found expressed as

per cent of larvae fed was distinctly greater than in the control animals. The respective percentages were: thiamine 79, controls 67; riboflavin 84, controls 80, with a mortality of eight-tenths in the deficient group; vitamin A 87, controls 72, and 95 and controls 71.

Resistance against superinfection was tested by giving a second large number of larvae a few days after the original infection and subsequently counting the worms found in the intestine twelve days after the second exposure. The percentage of total larvae fed found as worms in the deficient and the control animals was as follows: thiamine 25, controls 10; riboflavin 60, controls 5.6.

Tests for resistance to subsequent reinfection were made. Normal rats were hyperimmunized by means of serial infections. Three weeks after the last infection one half of each group was placed on one of the various deficient diets. As soon as the decreased growth rate indicated the establishment of the deficiency, the animals were tested and evaluated by infection and count as previously described. The percentage counts for the deficient and control animals were as follows: thiamine 5.1, controls 0.55; vitamin A 8, controls 0.6, and 10 and controls 0.7.

It has been demonstrated that the sera from hyperimmunized rats when given to normal rats could protect them from infection with N. muris. Hyperimmune serums were prepared in normal rats and in those deficient in the various vitamins being studied. The immunologic potencies of these serums were tested by giving 5 ml. intraperitoneal doses to normal rats which had just received an infecting dose of 500 N. muris larvae. The test animals were killed twelve days later and counts made as previously described. The percentage infection for animals receiving serum from deficient and control groups was as follows: thiamine 48, controls 23; riboflavin 44, controls 25; vitamin A 53, controls 28.

These thorough and well controlled experimental studies amply confirm the fact that inadequate nourishment lowers the resistance to parasitic infections. Furthermore, it seems clear that this lowered resistance can result from any one of a number of deficiencies—that it is not a specific mechanism which is involved, but rather the general biologic condition of the host.

THIAMINE REQUIREMENTS AND THE BREAD PROBLEM

Two rather recent reviews (McHenry, *Canad. Med. Assn. J.* **52,** *147 (1945)*; Holt, *Federation Proceedings* **3,** *171 (1944)*) have challenged the recommended dietary allowances for thiamine proposed by the National Research Council. McHenry has assumed that the thiamine requirements per 1000 nonfat calories are the same for various species of animals. He has then correlated the thiamine requirements as reported by several investigators with the percentage of total calories from fat. Such a treatment resulted in the suggestion that the minimal requirement for thiamine on a fat free diet is 0.19 mg. per 1000 calories. On a diet in which fat constituted 40 per cent of the total calories, the minimal requirement is placed at 0.17 mg. per 1000 calories. A suitable "optimal" allowance for thiamine of 0.23 mg. per 1000 calories is suggested for the usual Canadian diet in which approximately 40 per cent of the calories are derived from fat. Such a figure is based on (1) unpublished observations on rats in the author's laboratory, (2) the report of Arnold and Elvehjem (*Am. J. Physiol.* **126,** *289 (1939)*) on dogs in which an optimal intake was found to be 75 micrograms of thiamine per 100 nonfat calories, and (3) the report of Keys, Henschel, Mickelsen, and Brozek (*J. Nutrition* **26,** *399 (1943)*; see *Nutrition Reviews* **2,** *140 (1944)*) that amounts of

thiamine in excess of 0.23 mg. per 1000 calories provided no additional benefit to their human experimental subjects during short periods. Such a standard was reported to serve as a satisfactory basis for estimating the adequacy of food intakes in a nutritional survey (Ferguson, Leeson, and McHenry, *Canad. J. Pub. Health* **36,** *66 (1944)*; see *Nutrition Reviews* **2,** *264 (1944)*). McHenry concludes that "the allowance for thiamine now recommended is much too large and that, because of the use of this excessive allowance as a dietary standard, a great deal of the alleged deficiency is really non-existent. I would like to make clear, however, that while I believe the recommended allowance could be markedly lowered, there is not sufficient information at present to decide accurately what the optimal requirement for humans really is." Similar conclusions have been reached by Holt (*loc. cit.*).

Since the contention that widespread mild thiamine deficiency exists is based largely upon the data secured in dietary surveys which have been evaluated in terms of the higher allowance of approximately 0.6 mg. of thiamine per 1000 calories, McHenry concludes that "claims of widespread thiamine deficiency" are probably artifacts. A corollary of this conclusion is that the thiamine content of bread should not be the major factor determining a bread policy. Numerous other considerations involved in establishing a bread policy along sound nutritional principles have been reviewed by Lepkovsky (*Physiol. Rev.* **24,** *239 (1944)*).

PARA-SPRUE

It is not surprising that with newer nutritional knowledge, some of the chronic conditions in poorly nourished and disease-ridden countries should be recognized as nutritional deficiency conditions or that they should be at least complicated by such deficiencies. Chaudhuri and Chaudhuri (*Indian Med. Gaz.* **79,** *404 (1944)*) have described a syndrome in India as para-sprue: "A condition of chronic diarrhea associated with anemia and sore mouth, suggestive of sprue, has long been known in this country, and it is usually regarded as a chronic deficiency state." The patients complained of indigestion, flatulence, loss of appetite and strength, and showed a glossitis, macrocytic anemia, emaciation, and diarrhea. Diagnostic features of true sprue include (1) steatorrhea (which must be established by laboratory analysis showing over 30 per cent of fat), (2) marked loss of weight, (3) low glucose tolerance curve, (4) macrocytic and hyperchromic anemia, and (5) hypochlorhydria or achlorhydria. The authors note certain distinguishing features of "para-sprue" from a true sprue condition: It occurs among Indians "who rarely suffer from true sprue, the type of diarrhea and nature of the stool is usually different, extreme degrees of emaciation may be absent, and improvement occurred on parenteral administration of crude liver extract without strict dietary regime."

Cook (*Indian Med. Gaz.* **79,** *429 (1944)*) described what is undoubtedly the same syndrome and demonstrated that the condition is in large part a niacin deficiency. His paper is based on the clinical details of 50 cases. The chief signs were glossitis, skin pigmentation, macrocytic anemia, sore mouth, and diarrhea. The latter is described as resulting in copious, light-colored stools, passed irregularly, and accompanied by exhaustion. The author distinguishes the condition from true sprue largely on the character of the stools; in the condition studied the stools only occasionally showed excessive fat. The treatment recommended was to give niacin orally, and also parenterally if necessary. This generally controlled the diarrhea and the

accompanying dehydration, particularly in those patients who had an "acute relapse" with fiery red tongue. Further treatment with baker's yeast, peanut butter, wheat bran, liver extract, and iron is recommended to supply the whole vitamin B-complex and the hemopoietic factors of liver.

An acute, relapsing form of this disease was also described by Cook. This involved the appearance of a fiery red mouth, too sore to permit the patient to eat or even to drink, and a severe and bloody diarrhea. This syndrome responded within twenty-four hours to niacin therapy, and the author concluded that this syndrome is entirely attributable to vitamin B-complex deficiency.

In view of the classic observation of Goldberger and Tanner (*Pub. Health Reports* **39,** *87 (1924)*) that milk and buttermilk possess pellagra-preventive activity, it is of interest that the native treatment for this disease has been to restrict the diet to buttermilk and curds. Among a population where, for religious and economic reasons, many people eat little or no meat, the culture of the peanut and the growth of food yeast on sugar cane juice might be a feasible and effective means of providing inexpensive foods which would prevent this syndrome.

METHIONINE AND CYSTINE DEFICIENCY AND RELATIONSHIPS

When Osborne and Mendel (*J. Biol. Chem.* **20,** *351 (1915)*) showed that the growth rate of rats on an insufficient casein ration could be increased by the addition of cystine to the diet, it was concluded that cystine was an indispensable amino acid. The fact that methionine also could increase the growth rate when added to such a ration (Jackson and Block, *Ibid.* **98,** *465 (1932)*) rendered the position uncertain, and it remained for Womack, Kemmerer, and Rose (*Ibid.* **121,** *403 (1937)*) to demonstrate by the use of diets containing amino acid mixtures instead of proteins, that cystine is dispensable and methionine indispensable in the diet. This conclusion, gained from experimentation with the rat was found to pertain also to man. Thus Rose, Haines, Johnson, and Warner (*Ibid.* **148,** *457 (1943)*) found that in an experiment of six days' duration in a man, omission of methionine from the diet caused a negative nitrogen balance whereas omission of cystine failed to do so. In related experiments Albanese and co-workers (*Bull. Johns Hopkins Hosp.* **74,** *308 (1944)*) have studied methionine and cystine deficiency in man. In these experiments, control observations were made on 2 normal male subjects fed a diet supplemented with cystine and methionine. When these amino acids were withdrawn from the diet, otherwise deficient in these substances, a negative nitrogen balance promptly occurred. During the following thirty-six day period, a continuing negative nitrogen balance and loss of weight occurred, although no significant changes were noted in the plasma protein, hemoglobin, or blood cell counts. Addition of methionine to the diet (18.8 mg. dl-methionine per kilogram body weight) at the end of this period produced a prompt restoration of the nitrogen balance. This suggests that the entire human requirement of sulfur amino acids can be met in the normal individual by methionine alone.

These experiments indicated that cystine could be derived from methionine in the body and that the sulfur of the methionine might be a chief source of the sulfur found in cystine. This relationship was demonstrated by the use of radioactive sulfur in the experiments of Tarver and Schmidt (*J. Biol. Chem.* **130,** *67 (1939)*). The cystine prepared from the hair of rats fed methionine containing radioactive sulfur, proved that some of the sulfur of methionine, but not of inorganic sulfate, was used in the formation of cystine in the body.

The reactions by which the sulfur could

be transferred from methionine to cystine remained unknown, and even now are not established with certainty. A possible mechanism had been suggested by Brand, Block, Kassell, and Cahill (*Proc. Soc. Exp. Biol. Med.* **35,** *501 (1936–37)*). As a result of feeding tests with other compounds, these workers were led to suggest that homocysteine formed by the demethylation of methionine might react with amino-acrylic acid to give carboxyaminopropyl-S-cysteine, or cystathionine. This compound might then break down with the liberation of cysteine. Cystathionine was investigated in feeding trials by du Vigneaud, Brown, and Chandler (*J. Biol. Chem.* **143,** *59 (1942)*), who obtained evidence that it could replace cystine but not methionine or its demethylated derivative homocysteine in the diet. Their evidence of course did not establish that cystathionine is a normal metabolite, but did show that if it occurs in the body it may be expected wholly or in part to break down to cysteine. This evidence was reinforced when Binkley, Anslow, and du Vigneaud (*Ibid.* **143,** *559 (1942)*) isolated crystalline cystine after incubating a solution of cystathionine with a saline extract of fresh rat liver.

New evidence appeared however when Binkley and du Vigneaud (*J. Biol. Chem.* **144,** *507 (1942)*) showed that similar formation of cysteine by rat liver preparations occurred when they were incubated with homocysteine in the presence of serine. In the absence of serine much less cysteine was formed. Simultaneously Stetten (*Ibid.* **144,** *501 (1942)*), using again the technic of labeled atoms (heavy nitrogen), found that when rats were fed isotopic dl-serine for three days and then killed, their body proteins yielded isotopic cystine. These findings suggest that cysteine may be formed in the body from homocysteine, derived from methionine and uniting with serine or a serine derivative to yield cystathionine. At any rate, the conservative conclusion drawn by Stetten, that "the carbon chain of serine is converted to cystine in the rat" seems to be firmly established by his experiments.

Another study dealing with the mode of production of cystine from methionine in the body was reported by du Vigneaud, Kilmer, Rachele, and Cohn (*J. Biol. Chem.* **155,** *645 (1944)*). These authors again made labeled methionine, but this time they synthesized methionine containing not only heavy sulfur, but in addition heavy carbon in the carbon chain. The synthesis was suitably modified to obtain the best yield of methionine from the elementary sulfur, and the feeding experiment was devised to obtain the greatest production of cystine from methionine. The basal diet contained sucrose, Crisco, salts, vitamins and mixed amino acids, but no methionine or cystine. After three days on this diet the young growing rats were dehaired, and one group received the basal diet + 0.6 per cent labeled methionine, while a second group received the basal diet + 0.6 per cent ordinary methionine. The methionine content of the diets was reduced during the progress of the experiment in order to conserve the labeled methionine. After thirty-eight days (and two days on the basal diet only), the hair was washed, degreased, hydrolyzed, and the cystine separated from the hydrolysate by the method of Graff, Maculla, and Graff (*Ibid.* **121,** *81 (1937)*).

After purification and identification, a portion of the cystine was oxidized for the production of carbon dioxide and another portion was reduced for the production of hydrogen sulfide. These compounds were analyzed in the mass spectrometer. The hydrogen sulfide was found to contain isotopic sulfur, while the carbon dioxide contained no isotopic carbon. Thus it was shown again that the sulfur of methionine appears in cystine formed in the body, and it was shown conclusively that none of the carbon of the methionine molecule is found in the cystine.

Results of the investigations described

above may be summarized as follows: The cystine which is formed within the body of the rat contains a carbon skeleton which is derived from serine, and a sulfur atom which is derived from methionine. It is probable, but not proved, that the cystine results from a reaction between serine and homocysteine, the latter being derived from methionine. These compounds may react with production of cystathionine, a compound of thio-ether type from which cysteine can be split off in the liver. However, neither cystathionine nor homocysteine has been shown to be a normal metabolite in the animal, and until such proof is forthcoming, intermediate phases of the methionine to cystine scheme remain only tentative.

WERNICKE'S DISEASE

The history of ideas about the symptom complex known as Wernicke's disease typifies the changes which have occurred in medical thought about the manner in which addiction to alcohol produces its several evil effects on the victim. The first reported victim of this disorder had a long period of vomiting which suggested a nutritional factor (Wernicke, *Lehrbuch der Gehirnkrankheiten für Aerzte und Studirende* **2,** *229 (1881)*) but this idea was lost to view as increasing numbers of cases appeared in which the obvious conditioning factor was chronic alcoholism. In fact Wernicke's second and third cases occurred in patients with chronic alcoholism. There is modern evidence that many of the disease conditions formerly thought to result from alcohol as a poison are really the effect of a deficiency of special factors in the diet, first suggested for alcoholic polyneuritis (beriberi), and then pellagra. More recently the discovery of characteristic cerebral lesions occurring in experimental animals on thiamine deficient diets (Zimmerman, *Yale J. Biol. Med.* **12,** *23 (1939)*; Alexander (*Am. J. Path.* **16,** *61 (1940)*; and Evans, Carlson, and Green, *Ibid.* **18,** *79 (1942)*) has strongly suggested that Wernicke's disease belongs in this category also (*Nutrition Reviews* **1,** *24, 61 (1942)*). This idea was advanced by Jolliffe, Wortis, and Fein (*Arch. Neurol. Psychiat.* **46,** *569 (1941)*) on the basis of clinical experience with both alcoholic and non-alcoholic cases. Riggs and Boles (*Quart. J. Studies on Alcohol* **5,** *361 (1944)*) have presented further confirmation of this idea in a review of 42 cases of Wernicke's disease observed in the Philadelphia General Hospital during the ten years ending with 1943. Such a large series of cases offered an opportunity to clarify not only the etiologic background but also the clinical picture.

The diagnosis in all 42 cases was made at autopsy. The clinical manifestations varied and the syndrome could not have been established on clinical evidence alone in many instances. Psychic function was disturbed in all, usually initially, and 70 per cent had other signs of central nervous system involvement. The classic syndrome as described by Wernicke did not appear in its entirety but it must be remembered that the gravely ill patient with Wernicke's disease often does not satisfy the criteria for an adequate neurologic examination. Where agitation, confusion, and convulsions occur the examination is frequently cursory. The full blown syndrome is general agonal. It is of great interest to compare this series of cases which were diagnosed by autopsy findings with those reported by Jolliffe, Wortis, and Fein which were diagnosed on the basis of the clinical findings. The latter group depicted a clearcut pattern which was the basis of diagnosis whereas Riggs and Boles show clearly that the typical lesion of the central nervous system may be associated with a highly nonspecific clinical picture. The rather low incidence of periph-

eral neuropathy suggests that thiamine deficiency alone is not the complete cause of Wernicke's syndrome. Differences in clinical findings are inevitable when the criteria of selection of cases are different and this must be kept in mind when comparing these two studies. The data of Riggs and Boles certainly make desirable a further investigation of the role of various vitamins in the pathogenesis of this syndrome.

There were 25 white patients, 56 per cent being women, while 88 per cent of the Negro patients were women, mostly under 40 years of age. The group is far too small to evaluate as a fraction of the whole population, but there is nothing against the probability that a large proportion came from the low income, poorly fed group. There were many other reasons to implicate general and specific malnutrition. Only 18 gave histories or indications of chronic alcoholism but 31 had fatty livers, 15 had peripheral neuropathy, and others had extreme emaciation, edema, pellagrous dermatitis, stomatitis, and cheilosis in declining frequency. A number of complicating systemic diseases have been interpreted by Riggs and Boles as merely aggravating the severity of the depletion rather than initiating it. It is possible that acute disease processes precipitate the acute changes of Wernicke's disease but it is even more likely that the chronic diseases were well established conditioning influences. Acute infections were the most frequent precipitating conditions and protracted vomiting ranked second.

There is a detailed tabulation of the location and types of lesions found in various portions of the central nervous system, and the criteria for the diagnosis are presented. There was an interesting lack of absolute correlation between the lesions described in the brain and the clinical findings of extraocular palsies. In 4 patients with these palsies there was characteristic implication of the appropriate cranial nerve nuclei. Five presented palsies equally definite but there were no diagnostic lesions in the central nuclei. This fact emphasizes the disturbance of function before occurrence of typical lesions, a phenomenon common to all vitamin deficiency syndromes.

It would appear from the accumulated clinical studies that the case for the deficiency pathogenesis of Wernicke's disease is good. The lack of an obvious correlation with any syndrome recognized as originating from a specific deficiency may be explained by several possibilities which remain to be investigated. Wernicke's disease (1) may result from lack of a single factor, not yet identified, but presumably associated with the B-complex vitamins; (2) may arise from a multiplicity of deficiencies which must occur in some special combination or arise in some particular sequence; (3) the neurovascular lesions may result from interference with the function of some organ, such as the liver, allowing hormones or other agents to produce tissue changes; or (4) there may be a failure to detoxify products of metabolism or a reduction or absence of the formation of substances required for proper neurovascular health. Indeed the still unexplained association of cerebral and hepatic lesions in Wilson's disease (cirrhosis and progressive lenticular degeneration) might conceivably result from the operation of some obscure malfunction of the liver as a primary fault brought on by a specific deficiency.

These speculations about the mechanisms involved in the production of the typical lesions in the central nervous system in Wernicke's syndrome indicate the nebulous state of our knowledge. It can be improved only by painstaking collection of more clinical material examined with an open mind but keeping in view the strong testimony that some form of nutritional deficiency is involved.

THE TYROSYL TOLERANCE TEST IN PERNICIOUS ANEMIA

The fact that tyrosine increases the efficacy of some liver extracts in the treatment of pernicious anemia (Jacobson and Subba-Row, *J. Clin. Invest.* **16,** *573* (*1937*)) indicates that it may play a fundamental part in the metabolism of the factors whose proper interactions prevent the occurrence of pernicious anemia and whose prevented function determines its occurrence and degree. Further testimony of an abnormal state of tyrosine metabolism in pernicious anemia is suggested by the high levels of blood and urinary phenols which are found. Swenseid and Bethell (*Proc. Central Soc. Clin. Res.* **17,** *40* (*1944*)) have reported an investigation of the metabolisn of tyrosine in pernicious anemia based on the tyrosyl tolerance test of Bernhart and Schneider (*Am. J. Med. Sci.* **205,** *636* (*1943*)). This test has been carried out on patients with pernicious anemia before treatment and after remissions were induced by liver therapy. Comparisons were made with other varieties of anemia. The tyrosyl tolerance test consists of hourly determinations of the blood tyrosyl level after 4 g. of l-tyrosine have been given by mouth. It was used originally as a test of liver function. In normal persons the peak concentration of tyrosyl in the blood is obtained one hour after ingestion of tyrosine. In contrast, patients with pernicious anemia in relapse do not have a peak level until three hours after tyrosine is given. Following successful therapy, however, the curves obtained are normal and have a peak at the end of one hour.

Comparison of the course of blood tyrosyl levels in patients with pernicious anemia in relapse and patients with cirrhosis reveals that the latter have markedly elevated levels. This difference between cirrhosis and untreated pernicious anemia is not found in the bromsulfalein, hippuric acid, or galactose tolerance test which commonly show abnormalities in liver function in pernicious anemia in relapse as well as in cirrhosis.

The authors conclude that the tyrosyl tolerance curves in pernicious anemia may best be explained by a delay in absorption of tyrosine from the alimentary canal, this delay being corrected by the changes which accompany the specific correction of the anemia.

It would be of interest to have data on the alimentary absorption of other test compounds at the same stage of relapse at which the tyrosyl tolerance test was carried out. Critical analysis of the faulty metabolism might be achieved by a comparison of the fate of tyrosine injected intravenously with its fate when given by mouth.

RELEASE OF A THIAMINE-LIKE SUBSTANCE FROM NERVES DURING STIMULATION

Symptoms of impaired nerve function, such as peripheral neuropathy and opisthotonos, are prominent features in experimental thiamine deficiency in a number of species. Peters (*Lancet* **I,** *1161* (*1936*)) in classic experiments has shown that the carbohydrate metabolism of brain tissue *in vitro* is markedly altered as a result of thiamine deficiency. The question naturally arises as to whether the effects of thiamine on the nervous system can be wholly explained on the basis of the vitamin being a part of thiamine pyrophosphate, which is the coenzyme of a number of metabolic reactions, or whether thiamine in addition to this function exerts other effects on the nervous system. For instance, it has been suggested by several investigators that thiamine may play a direct role in the formation of acetylcholine. Ever since

Loewi's discovery of acetylcholine liberation during stimulation of the vagus nerve, physiologists have been searching for other active chemical substances which might be liberated during nerve stimulation.

Muralt (*Nature* **154,** *767* (*1944*)) has reported that stimulation of the vagosympaticus supplying the frog heart leads to the liberation of a diffusible factor which behaved chemically like thiamine, that is, it could be oxidized by alkaline potassium ferricyanide to a blue fluorescing substance extractable with butanol. That the substance liberated during the vagus stimulation was a thiamine compound was further substantiated microbiologically. Phycomyces blakesleeanus grew well on heart fluid collected during stimulation periods, but grew little or not at all on heart fluids from resting periods of equal duration. Comparison of the growth curves with thiamine solutions of known strength indicated that each milliliter of heart fluid from the stimulated periods contained about 0.01 microgram of thiamine. Microthiochrome determinations gave the same result.

Polarographic records of the heart fluid showed a pattern characteristic for extracts from nerves frozen in the excited state. Since ordinary thiamine cannot be detected by this method in amounts smaller than 10 micrograms per milliliter, the author concludes that the substance liberated from stimulated nerves is not identical with thiamine.

Acetylcholine and the thiamine-like substance were also determined during nerve degeneration. Acetylcholine disappears rapidly after section of the nerves while the concentration of the thiamine-like substance decreases more gradually, some being present even after seventy-two hours of degeneration. Degeneration could be protracted by the administration of thiamine.

The author concludes that the experiments described confirm the concept that acetylcholine formation is essential for the excitation or recovery process, and that thiamine is a reservoir substance closely connected with the formation and disappearance of acetylcholine. It must be borne in mind, however, that during stimulation of a nerve a number of substances are released due to changes in the permeability of the cell wall. The fact that a certain substance is released from a nerve during stimulation does not prove that this substance is connected with the more specific processes taking place in nerves. Furthermore, although Muralt has demonstrated the release of thiamine on stimulation of the isolated heart, he has not established whether this thiamine came from nerve or muscle. In the case of the polarographic data on heart fluid or nerve extract, it is perhaps permissible to question the validity of this method for identification of substances of unknown nature, such as a hypothetical thiamine derivative.

The specific role of acetylcholine in the transmission of nerve impulses at the synapsis is undisputed. However, it will be necessary in some other way to demonstrate that thiamine exerts a specific effect on the nerve function before definite statements can be made about its primary function in the nervous system.

THIAMINE REQUIREMENTS AT HIGH TEMPERATURES

The question of a possible change in the thiamine requirement of man with variations in environmental temperature has become of great practical interest since the United States armies have been fighting on widely separated fronts having vastly different climates. In particular the question had been raised, in view of earlier animal experiments, whether the thiamine requirement is increased under tropical conditions. A

paper by Kline, Friedman, and Nelson (*J. Nutrition* **29,** *35* (*1945*)) adds greatly to our knowledge of this subject and appears to clarify the question considerably.

It is well known that the thiamine requirement increases as the metabolic rate increases, therefore it might be expected that since less food is consumed in a tropical than in a cold climate, the thiamine requirement might actually be decreased. Horst, Mendel, and Benedict (*J. Nutrition* **3,** *177* (*1930*)) and Herrington (*Am. J. Physiol.* **129,** *123* (*1940*)) have reported the depressing effect of increase in the environmental temperature upon the total metabolism of the rat.

Mills (*Am. J. Physiol.* **133,** *525* (*1941*)), however, worked with rats kept in rooms at two temperatures (65°F. and 91°F.) and found, by varying the amount of thiamine in the otherwise constant basal diet, that at the lower temperature the food intake and weight increase of the rats were not further increased by raising the thiamine content of the diet above 0.6 mg. per kilogram of food, while at the higher temperature food intake and weight increase did not reach maximum levels until the thiamine content of the diet was raised to 1.2 mg. per kilogram. At the higher temperature the maximal food consumption was about 75 g. per week and the weight increase 130 g. in six weeks; at the lower temperature maximal food intake rose to 130 g. per week and growth to about 180 g. in six weeks. From these results Mills concluded that the optimal thiamine requirement for the rat is twice as high when the environmental temperature is 91°F. as when it is 65°F., and that "protection against the severe effects of excessive heat is afforded by an accessory thiamine intake above the ordinary daily need."

Sarett and Perlzweig (*J. Nutrition* **26,** *611* (*1943*)) extended the results obtained by Mills, and showed that if there was a large amount of thiamine in the diet, the tissue laid down by rats growing in a room at 91°F. was twice as rich in thiamine as the tissue from rats given the same diet in a room at 75°F. When the thiamine content of the diet was just adequate at 75°F., however, the rats kept on the same thiamine level at 91°F. laid down tissues of low thiamine content. Sarett and Perlzweig considered that these results could not be interpreted as evidence that thiamine requirement depends upon the environmental temperature, because the divergence in tissue thiamine levels with the different levels of thiamine in the diet was not correlated with the amounts of thiamine excreted in the urine.

Kline, Friedman, and Nelson, in the course of their studies on biologic methods for the assay of thiamine, both with the rat-curative and the rat-growth methods, observed a profound effect of environmental temperature on the thiamine requirement, necessitating careful temperature control in the rat rooms if accuracy is to be obtained, especially in the rat-curative assay. In their work, basal diets devoid of thiamine were used, and all thiamine received by the rats was given separately by stomach tube. In this way the thiamine intake was kept independent of the food intake, in contrast to the earlier experiments described above where the thiamine was mixed in the diet so that its intake depended upon the food intake. In rat-curative experiments they allowed rats to consume the thiamine-free basal diet ad libitum until polyneuritis developed. Each rat was then housed in a separate cage in a room maintained at constant temperature and given a single dose of 6 micrograms of thiamine hydrochloride to cure its polyneuritis. The time which elapsed until the polyneuritis recurred was recorded. In this way it was found that 6 micrograms of thiamine hydrochloride protected a rat from polyneuritis, on the average, for 8.7 days in a room at 78°F., and for 12.4 days in a room at 85°F.

In another experiment 10 rats went through four successive curative periods, at 78°F. for the first, third, and fourth periods,

and at 90°F. for the second period. In the first period at 78°F., 6 micrograms of thiamine hydrochloride produced cures averaging 9.1 days; in the second period at 90°F. half the dose, 3 micrograms, produced cures averaging 11.6 days; in the third period at 78°F., 6 micrograms again gave cures averaging 9.9 days; and in the fourth period, at 78°F., 3 micrograms produced no cure in 4 of the rats and cures lasting from 2 to 4 days in the other 6 rats. These experiments appear to show conclusively that as the environmental temperature of the rat is increased, its daily requirement for thiamine is decreased.

This conclusion is reinforced by the results obtained by the authors in rat-growth experiments, when the rats received a suboptimal amount of thiamine. When the rats were given optimal amounts of thiamine the food intake and growth response were directly controlled by the environmental temperature and therefore could not be related to the thiamine intake in any way enabling conclusions to be drawn about the requirement. On the other hand, when suboptimal amounts of thiamine were given, the thiamine intake became the limiting factor controlling food intake, and under these conditions it was clearly shown that with a given daily intake of thiamine more rapid growth occurred at 90°F. than at 78°F. It is pointed out by the authors that the failure of Mills, and of Sarett and Perlzweig, to reach this conclusion was due to the fact that they based their reasoning on results obtained with rats receiving an optimal amount of thiamine. For the groups of rats reported by Mills to have received suboptimal amounts of thiamine, it is remarked that calculation of the actual thiamine intake from the figures given for food consumption shows that rats kept at the higher temperature made greater weight gains on less thiamine than those kept at the lower temperature, which is in complete accord with the conclusions of Kline, Friedman, and Nelson.

The work of Kline, Friedman, and Nelson appears to clear up a number of difficulties affecting the experimental solution of this problem, and to show that the rat requires less, not more, thiamine at higher environmental temperatures. It will be presumed widely, until further experiments are reported, that the same is true of man.

DIET AND CHANGE IN WEIGHT

There is a persistent popular belief that some individuals gain in weight although they eat very little in comparison with energy expenditure, while others fail to gain despite a more than adequate food consumption. The facts, however, are to the contrary. It has become more and more certain that gain or loss in weight is determined over long periods solely by the relationship of caloric intake to caloric output. The search for a metabolic abnormality to explain obesity has been conspicuously unsuccessful. Careful investigations have shown that obese individuals have a total heat production which is even greater than normal and that they must, therefore, eat more than normal individuals merely to avoid loss of weight. It has been shown, further, that patients suffering from "endocrine" obesity consume amazingly large quantities of food exactly as do patients with obesity ascribed to other causes.

The relationship of calory surplus or deficit to weight gain or loss does not necessarily hold for short term experiments. A common clinical experience has been that individuals on low energy diets may not begin to lose weight for the first week or even longer. Newburgh and Johnston (*J. Clin. Invest.* **8**, *197 (1930)*) made a carefully controlled study of this point and showed that obese patients, despite a large calory

paper by Kline, Friedman, and Nelson (*J. Nutrition* **29,** *35* (*1945*)) adds greatly to our knowledge of this subject and appears to clarify the question considerably.

It is well known that the thiamine requirement increases as the metabolic rate increases, therefore it might be expected that since less food is consumed in a tropical than in a cold climate, the thiamine requirement might actually be decreased. Horst, Mendel, and Benedict (*J. Nutrition* **3,** *177* (*1930*)) and Herrington (*Am. J. Physiol.* **129,** *123* (*1940*)) have reported the depressing effect of increase in the environmental temperature upon the total metabolism of the rat.

Mills (*Am. J. Physiol.* **133,** *525* (*1941*)), however, worked with rats kept in rooms at two temperatures (65°F. and 91°F.) and found, by varying the amount of thiamine in the otherwise constant basal diet, that at the lower temperature the food intake and weight increase of the rats were not further increased by raising the thiamine content of the diet above 0.6 mg. per kilogram of food, while at the higher temperature food intake and weight increase did not reach maximum levels until the thiamine content of the diet was raised to 1.2 mg. per kilogram. At the higher temperature the maximal food consumption was about 75 g. per week and the weight increase 130 g. in six weeks; at the lower temperature maximal food intake rose to 130 g. per week and growth to about 180 g. in six weeks. From these results Mills concluded that the optimal thiamine requirement for the rat is twice as high when the environmental temperature is 91°F. as when it is 65°F., and that "protection against the severe effects of excessive heat is afforded by an accessory thiamine intake above the ordinary daily need."

Sarett and Perlzweig (*J. Nutrition* **26,** *611* (*1943*)) extended the results obtained by Mills, and showed that if there was a large amount of thiamine in the diet, the tissue laid down by rats growing in a room at 91°F. was twice as rich in thiamine as the tissue from rats given the same diet in a room at 75°F. When the thiamine content of the diet was just adequate at 75°F., however, the rats kept on the same thiamine level at 91°F. laid down tissues of low thiamine content. Sarett and Perlzweig considered that these results could not be interpreted as evidence that thiamine requirement depends upon the environmental temperature, because the divergence in tissue thiamine levels with the different levels of thiamine in the diet was not correlated with the amounts of thiamine excreted in the urine.

Kline, Friedman, and Nelson, in the course of their studies on biologic methods for the assay of thiamine, both with the rat-curative and the rat-growth methods, observed a profound effect of environmental temperature on the thiamine requirement, necessitating careful temperature control in the rat rooms if accuracy is to be obtained, especially in the rat-curative assay. In their work, basal diets devoid of thiamine were used, and all thiamine received by the rats was given separately by stomach tube. In this way the thiamine intake was kept independent of the food intake, in contrast to the earlier experiments described above where the thiamine was mixed in the diet so that its intake depended upon the food intake. In rat-curative experiments they allowed rats to consume the thiamine-free basal diet ad libitum until polyneuritis developed. Each rat was then housed in a separate cage in a room maintained at constant temperature and given a single dose of 6 micrograms of thiamine hydrochloride to cure its polyneuritis. The time which elapsed until the polyneuritis recurred was recorded. In this way it was found that 6 micrograms of thiamine hydrochloride protected a rat from polyneuritis, on the average, for 8.7 days in a room at 78°F., and for 12.4 days in a room at 85°F.

In another experiment 10 rats went through four successive curative periods, at 78°F. for the first, third, and fourth periods,

and at 90°F. for the second period. In the first period at 78°F., 6 micrograms of thiamine hydrochloride produced cures averaging 9.1 days; in the second period at 90°F. half the dose, 3 micrograms, produced cures averaging 11.6 days; in the third period at 78°F., 6 micrograms again gave cures averaging 9.9 days; and in the fourth period, at 78°F., 3 micrograms produced no cure in 4 of the rats and cures lasting from 2 to 4 days in the other 6 rats. These experiments appear to show conclusively that as the environmental temperature of the rat is increased, its daily requirement for thiamine is decreased.

This conclusion is reinforced by the results obtained by the authors in rat-growth experiments, when the rats received a suboptimal amount of thiamine. When the rats were given optimal amounts of thiamine the food intake and growth response were directly controlled by the environmental temperature and therefore could not be related to the thiamine intake in any way enabling conclusions to be drawn about the requirement. On the other hand, when suboptimal amounts of thiamine were given, the thiamine intake became the limiting factor controlling food intake, and under these conditions it was clearly shown that with a given daily intake of thiamine more rapid growth occurred at 90°F. than at 78°F. It is pointed out by the authors that the failure of Mills, and of Sarett and Perlzweig, to reach this conclusion was due to the fact that they based their reasoning on results obtained with rats receiving an optimal amount of thiamine. For the groups of rats reported by Mills to have received suboptimal amounts of thiamine, it is remarked that calculation of the actual thiamine intake from the figures given for food consumption shows that rats kept at the higher temperature made greater weight gains on less thiamine than those kept at the lower temperature, which is in complete accord with the conclusions of Kline, Friedman, and Nelson.

The work of Kline, Friedman, and Nelson appears to clear up a number of difficulties affecting the experimental solution of this problem, and to show that the rat requires less, not more, thiamine at higher environmental temperatures. It will be presumed widely, until further experiments are reported, that the same is true of man.

DIET AND CHANGE IN WEIGHT

There is a persistent popular belief that some individuals gain in weight although they eat very little in comparison with energy expenditure, while others fail to gain despite a more than adequate food consumption. The facts, however, are to the contrary. It has become more and more certain that gain or loss in weight is determined over long periods solely by the relationship of caloric intake to caloric output. The search for a metabolic abnormality to explain obesity has been conspicuously unsuccessful. Careful investigations have shown that obese individuals have a total heat production which is even greater than normal and that they must, therefore, eat more than normal individuals merely to avoid loss of weight. It has been shown, further, that patients suffering from "endocrine" obesity consume amazingly large quantities of food exactly as do patients with obesity ascribed to other causes.

The relationship of calory surplus or deficit to weight gain or loss does not necessarily hold for short term experiments. A common clinical experience has been that individuals on low energy diets may not begin to lose weight for the first week or even longer. Newburgh and Johnston (*J. Clin. Invest.* **8**, *197 (1930)*) made a carefully controlled study of this point and showed that obese patients, despite a large calory

deficit, might not lose any weight for as long as sixteen days. The composition of the diet also, as well as its caloric content, has been shown to influence the speed of the weight response. Almost forty years ago, Benedict and Milner (*U. S. D. A. Office Exp. Sta. Bull.* **175** (*1907*)) compared diets of equal (and slightly inadequate) caloric value and demonstrated that diets high in fat caused a much more prompt loss in the weight of the subject than those high in carbohydrate. Some years later, Lyon and Dunlop (*Quart. J. Med.* (*New Series*) **1,** *331* (*1932*)) reported that in short term experiments the loss of weight on diets containing 1000 calories appeared to be inversely proportional to the carbohydrate content of the food.

Results such as these when noted by untrained observers may well be responsible at least in part for the popular misconception concerning the inevitability and universality of the results of overeating or of partial fasting. Failure to lose weight immediately also constitutes a psychologic hazard for persons on reducing diets, tending as it does to discourage them at the very outset.

The explanation of these anomalies in weight behavior was discovered almost as soon as the anomalies themselves. Benedict and Milner by careful balance studies showed clearly and strikingly that a failure to lose weight on a high carbohydrate diet was accompanied by a retention of water, while an excessive weight loss on a high fat diet was accompanied by a negative water balance. One subject in a work experiment had a continuous deficit of approximately 500 calories per day. During three days on a high carbohydrate diet, the body weight increased 270 g. and there was a large positive water balance. In the following three days, on a high-fat diet of slightly greater caloric content, there was a weight loss of almost 3 kg., of which approximately 93 per cent was due to loss of water. Newburgh and Johnston in their study of the effect of low caloric diets on the body weights of obese individuals, demonstrated that departures from the predicted weight were always accounted for by storage or loss of water. One patient lost no weight in sixteen days despite a daily deficit of over 1200 calories; during this period she retained 2672 g. of water. During the following nine days on the same diet she lost 4000 g. in body weight with a negative water balance of 2266 g.

Anderson (*Quart. J. Med.* (*New Series*) **13,** *27* (*1944*)) has made a further investigation of the influence of the composition of submaintenance diets on loss of weight in obese patients. In his first experiments he noted that, in confirmation of the results of earlier investigators, high fat diets apparently led to a much more rapid loss of weight than high carbohydrate diets. From a comparison of analytical figures, however, he discovered that besides the differences in the amounts of carbohydrate and fat, the diets also differed in salt content. The chloride content of the high carbohydrate diet was found to be 3.99 g. per cent as sodium chloride, while that of the high fat diet was 1.33 g. per cent. Anderson was able to demonstrate a large positive salt balance as well as water balance on the high carbohydrate diet. In subsequent experiments, comparison was made of high fat and high carbohydrate diets of equal salt content and of high carbohydrate diets of differing salt content. The results of these experiments indicated clearly that the excessive water (and salt) retention on the original high carbohydrate diet was due to its high content of salt rather than to its high content of carbohydrate. On diets of equally low salt content and equal caloric content, whether high in fat or high in carbohydrate, weight losses were identical. From these experiments and from further studies carried out on 22 patients with various clinical "types" of obesity, the author was able to reach the conclusion that the reaction to undernutrition followed a similar pattern in all of the individuals that were studied. Provided the diet given was low in salt, weight losses from the very beginning of the test periods could be predicted with a good degree of approximation.

NOTES

Letter to the Editor

Dear Sir:

It is unfortunate that the article in the January issue of Nutrition Reviews entitled, "Usefulness and Toxicity of Gelatin" cited our article (*Proc. Soc. Exp. Biol. Med.* **56,** *187 (1944)*) to substantiate the work of Robscheit-Robbins, Miller, and Whipple (*J. Exp. Med.* **80,** *145 (1944)*), reporting that gelatin was toxic when administered to dogs intravenously. We feel that there is no similarity between the experiments reported in the two papers.

Our experiments consisted of oral feeding of a high level of gelatin to immature rats that were presumably normal at the start of the experiment. Robscheit-Robbins, Miller, and Whipple gave gelatin intravenously to dogs that were both anemic and hypoproteinemic and, therefore, could not be considered normal at the outset of the experiments. Our animals showed no gross pathology and the only effect we reported was that of growth inhibition.

Large amounts of gelatin solutions prepared by us for intravenous administration, have been given to human beings and to dogs with no evidence of thrombosis or of toxicity (Brunschwig and Nichols, *Surgery* **16,** *923 (1944)*). These solutions contained no preservative, since other work has shown the undesirability of having phenyl mercuric borate in gelatin solutions. As suggested by the reviewer, we feel that the effects reported by Robscheit-Robbins may be due to the different type gelatin used.

Sincerely yours,
Stanley W. Hier,
Claire E. Graham,
David Klein,
The Wilson Laboratories,
Chicago 9, Illinois.

Postwar Direction of Food Processing

Reports from several parts of the country presage great expansion in food processing studies, particularly with the view of increasing the nutritive value of processed foods. The Massachusetts Institute of Technology has announced a far-reaching program of research in food production and the improvement of processing methods. Special instruction in this field will be offered, with particular opportunity to returning service men.

Announcement has been made of the postwar construction of a sizable Food Research Building at the New York State Agricultural Experiment Station, a unit which will include a spacious pilot plant to bring plant operations close to the research laboratory. The program at this institution is designed to improve the nutritional qualities of foods produced by conventional processing methods and to develop new procedures of processing for conserving food values, as well as to select and breed varieties of agricultural crops for their nutritive value. Cornell University trustees have also accepted a considerable sum of money for its School of Nutrition, "an organization in which the Colleges of Agriculture, Arts and Sciences, Engineering, Home Economics, and Medicine are cooperating to provide an integrated program of research and teaching in foods and nutrition," supplementing the common association of nutritional studies with purely medical objectives.

A similar food processing research unit with courses of instruction is being sought by the agricultural interests of the University of California. The quarterly Alumni Bulletin of the University of Chicago reports that the university plans to establish in the near future a Food Research Institute.

Prenatal Diet

Two stimulating reviews of the progress of work relating the state of the offspring with the prenatal diet of the mother have appeared recently. One of these (Warkany, *Milbank Memorial Fund Quart.* **23,** *66 (1945)*) summarizes the convincing evidence that induced dietary deficiencies of riboflavin and vitamin D in the pregnant rat lead to the appearance of congenital abnormalities in the young (see *Nutrition Reviews* **1,** *49, 207 (1942–43)*; **2,** *157, 297 (1944)*).

The second article (Burke, *Milbank Memorial Fund Quart.* **23,** *54 (1945)*) recounts many of the striking correlations which have been observed to exist between the maternal diet during pregnancy and the health of the baby (see *Nutrition Reviews* **1,** *276, 386 (1943)*). Thus, it was found that when the diet of the mother was rated as "good" or "excellent," 42 per cent of the infants were rated as "superior," and only 3 per cent fell into the "poorest" classification. In contrast, of the cases where the maternal diet was "poor to very poor" only 3 per cent of the infants received the "superior" rating and 67 per cent were in the "poorest" classification. Further, a significant relationship was found between protein intake by the mother and the birth length of her child—the longer children were born to mothers with the higher protein intake. It was concluded that "from a practical standpoint these results indicate that less than 75 g. of protein daily during the latter part of pregnancy result in an infant who will tend to be short, light in weight, and most likely to receive a low pediatric rating in other respects . . . with an inadequate prenatal diet the fetus may suffer to a greater degree than the mother."

Vitamin C Content of Wild Greens

Many wild greens or common weeds are edible and fit for human consumption, and indeed in pioneering communities the use of these materials for food has not been infrequent. A current interest in certain of the edible wild greens has been revived in connection with a search for local foods suitable for addition to the wartime diet, and Murray and Stratton (*J. Nutrition* **28,** *427 (1944)*) have determined the vitamin C values for ten different wild greens. They used the dye titration method of Loeffler and Ponting (*Ind. Eng. Chem. (Anal. Ed.)* **14,** *846 (1942)*) and tabulated the values not only for the fresh plants but also for cooked and frozen samples. For purposes of comparison, spinach was used as the standard reference material; spinach contains 0.5 to 0.65 mg. of vitamin C per gram (Booher, Hartzler, and Hewston, *U. S. D. A. Circular no.* **638,** *244 (1942)*). Pokeweed, tall dock, and curly dock were found to contain nearly twice as much vitamin C as spinach; povertyweed, shepherd's purse, and lamb's quarter possessed approximately the same vitamin C content as spinach; chickweed, the common dandelion, the red seeded dandelion, and prickly lettuce contained less vitamin C than the reference material.

The vitamin C content of wild greens appeared to be influenced by several factors such as soil, moisture, temperature, stage of development, and the time of day the sample was taken for analysis. There was also some indication that the vitamin C content was greatest in plants which were exposed to full sunlight and which were growing in fertile uncultivated soil. A more exhaustive and detailed study of the vitamin C values of such greens grown under carefully controlled conditions is necessary however before any general correlation of these factors with vitamin content can be drawn.

Recent Nutrition Books

Food for the World. Edited by Theodore W. Schultz. University of Chicago Press, Chicago 37, Ill. 1945. pp. 352.

Hidden Hunger. Icie G. Macy and Harold H. Williams. The Jaques Cattell Press, Lancaster, Pa. 1945. pp. 320.

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